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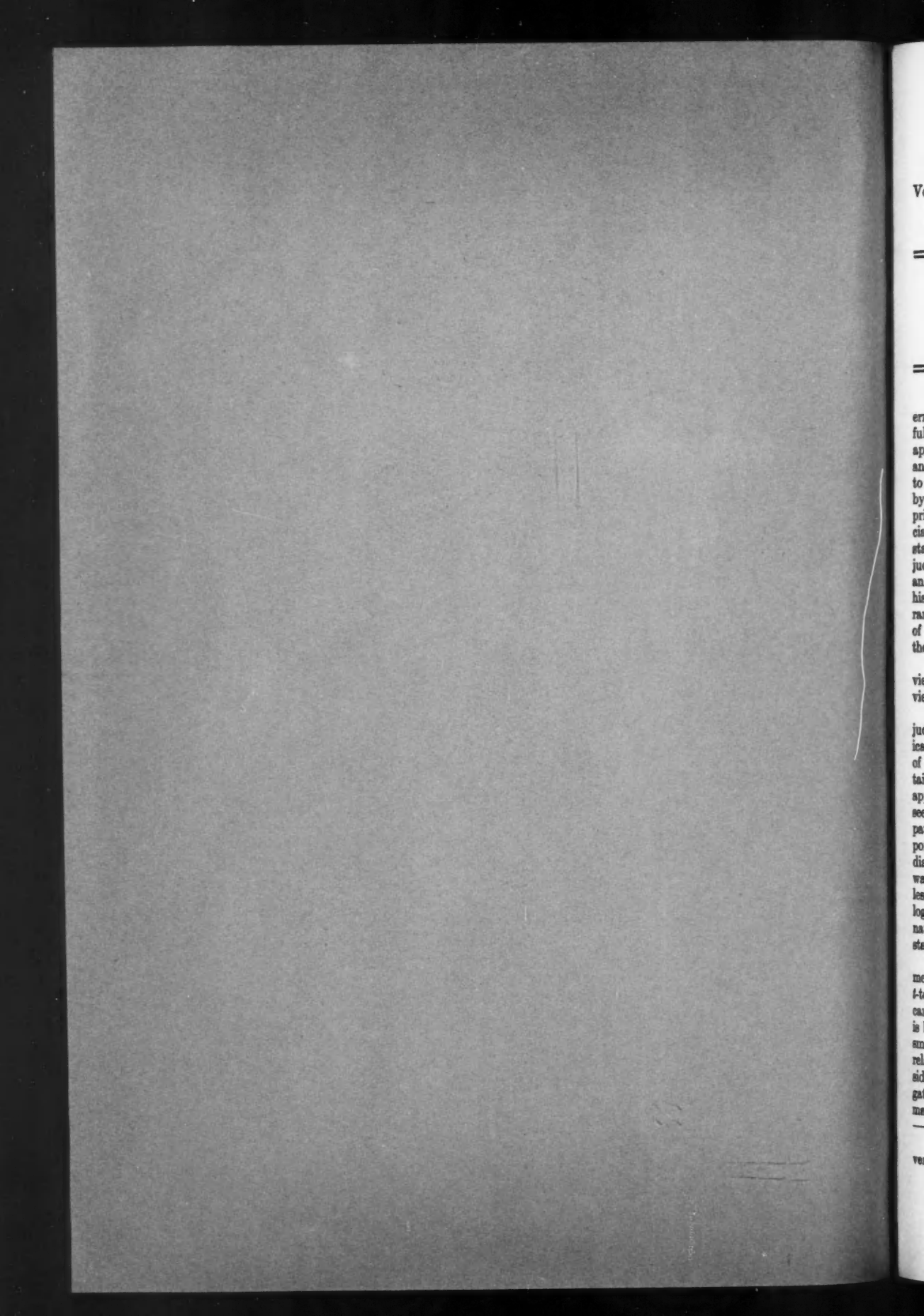

Vol. IV, No. 4 Clinical Research PROCEEDINGS November 1956

Statistics and the Individual Patient

By Eric Reiss*

AMONG THE MOST astonishing features of modern science of statistics is the ease with which powerful statistical methods can be employed. This ease of application has invited widespread use in research, and it has become customary for editors and readers to frown upon data whose validity is not sanctified by some statistical manipulation, however inappropriate it may be. It is therefore surprising that clinicians feel compelled to apologize whenever they use statistical reasoning in arriving at a diagnostic judgment, as though this sort of reasoning were an admission of utter failure. If the clinician bases his judgment in part on statistical information, he rarely does so without quickly stating his recognition of the fact that "statistics don't mean anything in the individual case."

In order to examine the validity of this point of view, a few basic statistical concepts must be reviewed.

In the final analysis, all diagnoses are statistical judgments. This is true of histologic as well as clinical diagnoses. One is reminded, in this connection, of a pathologist who diagnosed carcinoma of a certain organ on the basis of the characteristic histologic appearance. When, 15 years later, the patient was seen again, apparently in excellent health, the pathologist unhesitatingly obtained his original report and changed its wording to indicate a benign diagnosis. His concept of carcinoma of this organ was sufficiently rigid that he could not conceive of lesions having similar appearance but varying biologic behavior. It is the remarkable variation in the natural history of many diseases that invites a statistical orientation.

When an investigator compares two sample means by the appropriate statistical arithmetic, say a *t*-test, and concludes that the means differ significantly at a stated level of probability, his conclusion is based on *individual* samples. These are generally small in an absolute sense, and very small indeed in relation to the population of which they are considered to be random representatives. The investigator always recognizes that his particular sample may have been unusual, and the possibility of error due to sampilng is quantitatively expressed by the probability statement. If statistics were useless when applied to individual samples, the investigator would have to test the entire population. This is generally impossible. The overwhelming value of statistical technics is due to the fact that their intelligent application makes possible inductive reasoning from individual to population. Whatever the reasons for the difficulties in applying statistics in clinical diagnosis may be, they certainly have nothing to do with the clinician's concern with individual patients.

By a comparison of statistics as applied in the laboratory and the clinic, it is possible to bring the clinical difficulties into sharper focus. In the example given in the preceding paragraph, the test of significance could not prove that the samples were different. The test indicated only that differences as great as those observed would occur owing to chance variation in only rare samples. If chance is an unlikely explanation for the observed differences, other factors presumably produced them. The test of significance yields absolutely no information about what these factors may be. Such information is obtained only from the actual conduct of the experiment: if treatments X and Y were assigned at random to individuals drawn from a population, and uniform experimental procedure was observed throughout, some of the difference in effect between the X-treated and Y-treated individuals is probably due to differences between X and Y.

The investigator's advantages over the clinician may now be noted: (1) He can test an exact hypothesis. This is generally the so-called null hypothesis, which states that samples are random representatives of the same population, with factors X and Y having no real effect. This hypothesis can never be proved, but may be rendered very unlikely. (2) Having rejected the null hypothesis, the investigator possesses a knowledge about the cause or causes of observed differences from the way in which the experiment was conducted.

By contrast, the clinician generally obtains little diagnostic help from a test comparable to the test of the null hypothesis. He is interested in the causes of certain effects, and testing the null hypothesis—as the investigator's example illustrates—yields no information whatever about these. Suppose that

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factor *Z* has been measured in the serum of many normal persons, and that its distribution has been carefully determined. If a patient's serum is now tested for this factor, one may calculate the probability that the patient's serum could be a random sample of the population of normal sera. If this probability is very small, it may be concluded that the patient is abnormal as regards this factor. But if abnormal, what disease entity is suggested by the measured abnormality? To answer this question, information is required about all possible disease populations. The population of which the patient's serum is most likely to be a random sample is more strongly suggested than others. In practice, such reasoning is rarely helpful because abnormal populations tend to be rather alike, and, in general, too little is known about the distributional characteristics of abnormal populations. It should be noted that the present example is analogous to the preceding one; we are dealing here with a single serum, which is simply the mean of one serum. The investigator dealt with a single sample composed of several individuals, but the logical problem is the same in both instances: testing whether an individual could be considered to belong to a specified population.

These arguments may be summarized as follows: Both investigator and clinician deal with individuals chosen from very large populations. Having performed a well-designed experiment, the investigator can determine the probability that his samples came from a specified population. If this probability is very small, he rejects the null hypothesis and concludes that the samples probably belong to different populations. With this conclusion, he immediately knows from what populations the samples were probably derived. The clinician, on the other hand, knows little about the population to which his patient may belong after rejecting the null hypothesis. Probability-testing alone restricts both investigator and clinician to the negative conclusions that the individual sample or individual person is unlikely to belong to a specified population, though the degree of unlikelihood may be quantitatively assessed. The positive proposition that an individual does belong to a specified population defies quantitation.

The actual application of statistical reasoning in clinical medicine is not as difficult as these remarks may imply. In general, the diagnosis can usually be narrowed down to two or three major diagnostic possibilities, and it is the choice between them that taxes the clinician's skill and judgment. Consider a simple, idealized example: In a particular patient, all diagnostic aids point towards a choice between two diseases, but there are no clues to aid in the differentiation. If one of these diseases is known to occur twice as frequently as the other, the clinician has twice as good a chance of being right when he decides in favor of the more common disease. Of course, this sort of reasoning is correct only if (1) the frequency of occurrence of the diseases is actually known, and (2) there really are no discernible distinguishing features between the diseases. The essential point of the argument is that, given these conditions, the probability considerations can be definitely applied to an individual patient. The probability that the patient has one or the other disease cannot be estimated, but the relative probability can be obtained, at least in this idealized case. In practice, the true frequencies may be known only within rather wide ranges, and powerful diagnostic hints are often overlooked. The good clinician assiduously searches for these hints. Textbooks and the literature abound with data that partially characterize disease populations, and these data are extremely useful for the physician dealing with an individual patient. Documented experience thus supplies the valid basis for inductive inferences that result in a diagnosis. Insofar as such experience is properly obtained, it permits some quantitative probability estimates, and therefore comes within the proper domain of statistics.

There is an unfortunate tendency toward careless collection and interpretation of clinical statistical data. Clinical papers are often deplorably vague about the method of patient selection used. Patient populations vary widely between various localities.* Since reported data usually deal with small samples, population estimates derived from such samples are subject to large variation.

All these difficulties call for more, and not less, good statistics. Many excellent volumes have been written about the features of good statistics (which is the basis of proper induction) and the features of bad statistics (which is the basis of confusion and error). All that needs to be said here is that unbiased sampling from well defined populations is one of the most important features of good statistics.

The good, experienced clinician, of course, uses his own past acquaintance with disease as a statistical yardstick, even if he has never heard of samples, populations, induction and null hypotheses. However much one may resent it, diagnoses are statistical judgments. If these judgments are formulated purposefully and based on the experience of a large number of competent observers, so much the better and an apology is hardly in order.

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* The difference between populations is particularly striking in diagnostic exercises like clinicopathologic conferences. Here very unusual populations are sampled—difficult or unusual problems. Institutions vary in their preference for different kinds of cases. A physician is well justified in making a diagnosis at such a conference that he would not make at a bedside. After all, he is sampling an entirely different population, and a most unusual one at that.

Medical Research in the Area of the Southern Section of the American Federation for Clinical Research*

By John H. Moyer†

WITH THE DEVELOPMENT of new schools and the expansion of older ones throughout the south, many opportunities have become available for enterprising young men interested in medical research. Since I originated from an old traditional school—the University of Pennsylvania—and later moved to a relatively new medical center in Houston, I felt that a worthwhile topic for my discussion today would be medical research as seen through the eyes of a young investigator in the Southern Section of the AMERICAN FEDERATION FOR CLINICAL RESEARCH. Furthermore, I should like to make a few suggestions as to how I think this organization now serves, and might better serve, the younger clinical investigators throughout the South. The latter proposal is particularly pertinent since the AMERICAN FEDERATION FOR CLINICAL RESEARCH was conceived and organized for the purpose of stimulating research among young investigators in the clinical and allied medical sciences.

Medical research is a relatively new experience in many areas in this section of the country, and, therefore, the medical investigator is faced with problems which are entirely different in comparison to those present in established traditional institutions. The investigator, even though a novice, must develop his own program and pattern of activity, a responsibility usually entrusted only to the wise, mature and well-seasoned men in the traditional atmosphere. In many areas of the South the clinical investigator cannot depend on fixed administrative channels and old established rituals to support his activities. Usually he finds a shortage of medical personnel, rather than three teams for every job. He must adapt his program so that his research is not only investigative, but that it also offers something toward the medical care of heavy patient loads.

As a clinical investigator born and trained in the atmosphere of tradition, the following observations based on experience in a relatively new medical school stand out in my mind:

(1) The academician may easily find himself completely occupied with routine patient care. To achieve a better work balance requires organization, persistence, tenacity and drive toward maintaining the kinetics of a research program; otherwise, research is sacrificed and the academic clinician becomes a slave to full-time routine care of patients—a position which continues to be fostered by many medical school and hospital administrators.

(2) To keep a research program going requires adaptability on the part of the clinician. If obstacles arise in one area, it is necessary for the investigator to take the next avenue of approach, but he must keep driving continually. Although the program may at times move forward in a somewhat tangential course, at least it will move forward. One cannot afford to become frustrated and beaten, for, if this occurs, the program disintegrates in short order due to the absence of "traditional administrative channels and support." The same circumstances which foster opportunities for the young investigator in the untraditional centers become liabilities if the investigator loses his drive and kinetic approach.

(3) At the same time the investigator cannot afford to subjugate all efforts to his research aims. In the over-all program, patient care must exist if the investigator is to obtain cooperation from medical school and hospital administrators, as well as from the allied services. When there is a shortage in medical personnel, the investigator must give at the same time that he takes. It is not often possible for the clinician in this area to isolate himself to gadgeteering in the field of so-called "pure research."

(4) It seems to me that the best clinical research program is the one which combines research, patient care and teaching in a well-organized manner. The research program should be integrated into the over-all academic program. This program should include students, residents and staff men, not merely as props, but as "active characters in the play." In the research program, residents and students, when possible, should enter into the experimental design and the conduct of the experiment, and not simply act as the "guinea pigs" or subjects. This allows for stimulation and greater motivation of men who subsequently will form a reservoir of academicians and medical investigators. The AMERICAN FEDERATION FOR CLINICAL RESEARCH has most to offer to this latter group of investigators. Of necessity, numerous research projects will have to be designed for all levels of research experience. This will include projects which require high degrees of technical skill and experience, along with some well-designed, but not overly complicated experiments, so that the inexperienced investigators may develop insight into the problems under study.

* Chairman's Address, annual meeting of the Southern Section, American Federation for Clinical Research, New Orleans, January 1956.

† Baylor University College of Medicine, Houston, Texas.

(5) Finally, the responsible investigator should be cognizant of the fact that a research program is composed of men and women. One of the outstanding reasons for failure of research programs is our occasional inability to live with one another and appreciate our fellow-man. Research might better be looked on as a privilege. Let us be honest with our peers, and with ourselves, and use research in the quest for scientific truth rather than as an end to personal gain. If this quest be the prime motivating force, animosities among collaborators and contemporaries in research programs will be less in evidence. When research is a team enterprise, let each member obtain his just due, irrespective of his station in life, school appointment, rank, or what have you. When these principles are followed, team-mates in a research organization rarely strike out!

This brings me to the second point in this discussion. How can the AMERICAN FEDERATION FOR CLINICAL RESEARCH help the young investigator in the Southern Section?

Let me begin by outlining a brief background of this organization. The AMERICAN FEDERATION FOR CLINICAL RESEARCH was founded in May, 1940, but the first constitution was not adopted until 1947. A brief outline of the history of the organization is presented in the September 1955 issue of CLINICAL RESEARCH PROCEEDINGS by Dr. Beierwaltes, who was President at that time. The purpose of the organization is to provide a medium in which young investigators may get together and present and discuss the results of their research activities. The organization for such exchanges has been developed at three levels: (1) local research clubs, (2) four sectional groups (Eastern, Midwestern, Western and Southern) and (3) the annual (National) meeting. The total membership, which now exceeds 2500, is not to be limited. The national organization supports the publication of CLINICAL RESEARCH PROCEEDINGS, which is distributed to all members at a nominal cost.

Although the national organization has moved along rather rapidly, the Southern Section has not kept pace. This is owing largely to the fact that *lack of activity and responsibility among officers and members* has predominated, rather than organization, kinetics and activity. The latter, not the former, makes for a strong organization. This criticism I can apply without malice toward former officers of the section, since I am open to the same criticism, having been an officer of this Section for four years. How can we improve this situation? I believe that the following points are worthy of consideration, not only for the Southern Section but for other sections as well:

(1) *Program of the Southern Section:* papers for presentation should be selected on the basis of the following considerations:

(a) Excellence of research projects.

(b) Preference to young investigators for the presentation of papers. The members should keep this in mind when preparing abstracts. An individual should present only one paper at a meeting, and the paper should be presented by one of the younger authors whenever possible.

(c) A geographic distribution for selection of abstracts, in order to stimulate areas which are less active. If papers from one or two areas completely dominate the program, members from other areas which are new and less well-established become discouraged. Furthermore, the various areas which have many members have the option of setting up FEDERATION research clubs which will permit all of the investigators to present papers in those areas which have a large number of members. A number of such clubs has already been established in the Southern Section. When all areas have become strong and well-established, then excellence of the research project alone can be the sole consideration.

(d) A broad spectrum of papers, representing a variety of subject matter.

(e) Expanded meeting facilities. When the number of abstracts submitted for the annual meeting of the section exceeds by three times the number on the program, then two simultaneous panels should be set up or the meeting extended to two days.

(2) *A Policy and Membership Committee should be established with the Secretary-Treasurer of the Section acting as Chairman.* This should be a semi-permanent committee with each appointee serving several years on a staggered system. A replacement should be appointed each year by joint action of the Chairman and Secretary of the Section. A minimum of one member from each state should be on the committee. The members should be active throughout the year and should meet at the annual meeting of the Section. The Chairman of the committee should make a formal report each year at the business meeting. The purpose would be to develop policies and keep in contact with all geographic areas within the section in regard to ideas for improving the function of the organization, developing research clubs, and maintaining communication with active investigators and prospective members.

(3) *Improve liaison with the national organization.* It has become essential that the liaison and administrative channels be improved between the various sections of the AMERICAN FEDERATION FOR CLINICAL RESEARCH and the national organization. Let's make this a two-way organization! Presently, there is not much in common between the administrations of the national organization and the sections. There is a minimum of communication between the two. The sectional officers receive no reports from the annual council meeting or the business meeting of the national organization. There are no officers in common. The sectional chairmen attend one national council

meeting, but then retire, and there is no continuity of thought relative to organizational procedures and activities. The Chairman and Secretary-Treasurer of the national organization are selected from the national council members who are not selected by the section. Consequently, the officers are chosen by acquaintances rather than by sectional distribution, since the national officers are selected by a nominating committee, the members of which may have little if any relationship to or knowledge of the various geographic sections.

I would suggest that the council of the Southern Section consist of seven members: the Chairman, past Chairman, Secretary-Treasurer, the National Councillor of the Southern Section, and three Sectional Councillors. The Southern Section should select three sectional councillors to serve a term of three years, and one National Councillor to serve a term of five years. The national councillor should also serve as a fourth sectional councillor. An amendment should be made to the national constitution that national councillors must be elected from among the sectional councillors of the section. This will always mean that at least one individual will be serving at both the sectional and national levels. National councillors would serve a term of five years.

Reports of both the annual (National) council meeting and the annual business meeting should be made available to the officers of the various sections well in advance of the sectional meetings. This will allow items of business to be discussed which were brought before the national meeting and which are of importance to the sections.

NOTICES

Members Lost to the National Office

It would be appreciated if each member would review the following list and notify Dr. William W. Stead (V. A. Hospital, Minneapolis 17, Minnesota) of the current address of anyone on the list whom he recognizes:

W. J. Butt
Ronald T. Cape
Roy C. Crosby
Vincent J. Fontana
George L. Forbes, Jr.
Goffredo G. Gensini
Michele Gerundo
John F. Gillespie
Marvin M. Hirsch
Erwin Huston
Robert L. Johnson
Donald F. Marion
Mary L. Scholl
John R. Sheehan
Santos Silva
Bernard M. Wagner
Walter S. Wiggins
Irvin Zeavin

Forthcoming Meeting

The tenth annual meeting of the Western Society for Clinical Research will be held Thursday afternoon, Friday morning and Saturday morning, January 31st through February 2nd, 1957, at Carmel-by-the-Sea, California.

Information regarding the meeting may be obtained from Dr. Arthur J. Seaman, Secretary-Treasurer, Western Society for Clinical Research, University of Oregon Medical School, Portland 1, Oregon.

PROGRAM, MIDWESTERN SECTION

American Federation for Clinical Research

Thursday, November 8, 1956

Thorne Hall, Northwestern University, Chicago, Illinois

Dr. Harper K. Hellems, Presiding

Presentations will be limited to ten minutes

9:00 a.m.

1. **Nephrotic Syndrome in Adults.**
   Victor E. Pollak, Robert M. Kark,† Conrad L. Pirani and Robert C. Muehrcke.* Chicago
   page 250
2. **Changes in Body Water Compartments During the Course of Acute Renal Insufficiency.**
   *Alexander P. Remenchik, James A. Schoenberger and Josephine M. Dyniewicz.** Chicago.
   page 252
3. **The Ratio $\frac{Tm}{Renal\ Weight}$: An Index of Renal Scarring.**
   A. P. Crosley, Jr., J. F. Brown, B. Schuster,* D. A. Emanuel,* H. Tuchman, C. Castillo* and G. G. Rowe.* Madison, Wisc. *page 252*
4. **Relation of Renal Vein Pressure to Renal Vascular Resistance and Urine Flow Rate.**
   Francis J. Haddy, Malcolm Fleishman and Jerry Scott.** Fort Knox. *page 252*
5. **Cutaneous Necrosis Due to Norepinephrine: Mechanism and Prevention.**
   A. Stephen Close and Ross C. Kory.* Wood, Wisc. *page 241*
6. **Mechanism of Increased Serum Activity of the Enzymes, Glutamic-Oxaloacetic Transaminase (SGO-T), Glutamic-Pyruvic Transaminase (SGP-T), and Lactic Dehydrogenase (SLD), Following Myocardial Infarction in the Dog.**
   Irwin Nydick, Paul Ruegsegger, Felix Wroblewski and John S. LaDue.†* New York.
   page 240

INTERMISSION (10 Minutes)

Refreshments courtesy of G. D. Searle & Co.

7. **Nitrogen Clearance Rates of Right and Left Lungs in Different Postures.**
   Glen A. Lillington, R. Drew Miller, Ward S. Fowler† and H. Frederic Helmholz, Jr.†* Rochester, Minn. *page 256*
8. **A Rapid Colorimetric Method for the Determination of Oxygen Saturation of Whole Blood by Reflectance.**
   William Meltzer and George A. Saxton, Jr.* Chicago. *page 255*
9. **Endobronchial Involvement in Systemic Sarcoidosis.**
   *Gordon L. Snider and S. Allen Mackler.** Chicago. *page 257*
10. **Probable Mechanism of Ventricular Fibrillation and Cardiac Arrest Following Hypercapnia.**
    Ananda S. Prasad, E. B. Brown, Jr. and Edmund B. Flink.†* Minneapolis.
    page 239
11. **The Neutralizing Antibody Response to Adenovirus Infection.**
    J. Thomas Grayston, Clayton G. Loosli, Paul B. Johnston,* Mabel E. Smith* and Robert L. Woolridge.** Chicago. *page 250*
12. **Metabolic Interrelations of Calcium and Magnesium in Patients With and Without Osteolytic Disease.**
    William O. Smith and Leonard P. Eliel.* Oklahoma City. *page 245*

1:00 p.m.

LUNCHEON AND BUSINESS MEETING

Abbott Hall Dining Room

Courtesy of Eli Lilly and Company

2:00 p.m.

13. **The Effect of Bromine Ion on I^{131} Thyroid Function Studies.**
    *Richard E. Peterson and Masa Yamamoto.** Iowa City. *page 242*
14. **The Hyperglycemic Response as a Measure of Glucorticoid Potency.**
    Kelly M. West and James A. Hagans. Oklahoma City. *page 244*

* By Invitation
† Senior Member

15. **Comparative Effects of Insulin and Orinase on Blood Levels of Pyruvate and Alpha Ketoglutarate in Normal Subjects.**
Allen R. Hennes, Bernardo L. Wajchenberg, Stefan S. Fajans and Jerome W. Conn.** Ann Arbor. *page 242*

16. **The Effect of the Administration of an Intravenous Fat Emulsion Upon the Blood Lipids of Normal and Hospitalized Subjects.**
Jack M. Iacono, William W. Cleland,* Lucille Palm* and John F. Mueller.* Denver. *page 245*

17. **5-Nucleotidase Activity of Human Serum.**
Irving I. Young. Detroit. *page 246*

18. **Percutaneous Transhepatic Cholangiography.**
William T. Fitzgerald, James C. Redington* and William A. Knight, Jr.* St. Louis. *page 248*

INTERMISSION (10 Minutes)

19. **Hepatic Function and Morphology in Chlorpromazine Jaundice as Affected by Continued Administration of Chlorpromazine.**
Edward M. Schneider, Charles Daugherty and James K. DeVore.** Oklahoma City. *page 248*

20. **Significance of Increased Urinary Pepsinogen (Uropepsin) Excretion in Duodenal Ulcer and During ACTH Administration.**
B. I. Hirschowitz, D. H. P. Streeten and H. M. Pollard.† Ann Arbor. *page 247*

21. **C.I.B.H.A. Infuscusuria: A New Clinical Syndrome.**
*Robert D. Lange and Joseph H. Akeroyd.** Washington, D. C. *page 234*

22. **Homozygous Hemoglobin-D Disease.**
Amoz I. Chernoff, George Smith and Ruth Steinkamp.* St. Louis. *page 233*

23. **Cobalt-60 Labeled Vitamin B-12 Absorption and Excretion Studies Using Timed Release Capsules.**
*Philip C. Johnson and E. Stanley Berger.** Oklahoma City. *page 234*

24. **Erythrocyte Radioiron Uptake in the Rat and the Effects of Cobalt Administration.**
Donald R. Korst and Frank H. Bethell.† Ann Arbor. *page 232*

Officers of the Midwestern Section

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Advance Reports Submitted to the Annual Meeting of the

Midwestern Section

of the

American Federation for Clinical Research

Thorne Hall, Chicago, Illinois · Thursday, November 8, 1956

BLOOD

Long-Term Studies of Iron Overload in Dogs

By *Elmer B. Brown, Jr., David E. Smith, Reubenia Dubach, César Reynafarje and Carl V. Moore.* Department of Medicine, Washington University School of Medicine, St. Louis.

Large amounts of iron as saccharated iron oxide or as hemoglobin from transfused red blood cells were given to 6 dogs to determine the effects of prolonged iron-overload and in an attempt to produce hemochromatosis. Intravenously administered saccharated iron oxide was given in 100 to 200 mg. daily doses to a total iron concentration of 0.5 Gm./Kg. in 2 dogs and 1.0 Gm./Kg. in 2 others. Transfusions of whole blood totalling more than 12 L. were given to each of 2 dogs to yield concentrations of 0.5 Gm./Kg. hemoglobin iron. At intervals during a 7-year period of observation, blood counts, serum iron, fasting blood sugar, serum bilirubin, bromsulfalein retention and serum protein determinations were done. Tissue samples for iron analysis and histologic study were obtained by biopsy initially, at 6 months and 3 years after beginning iron administration, and at autopsy.

Despite concentrations of iron chosen to be comparable to those found in human hemochromatosis, there were no findings in the dogs suggestive of hemochromatosis. Acute toxicity was minimal in spite of greatly elevated serum iron levels, and no laboratory evidence of cirrhosis or diabetes was obtained. Blindness due to retinal degeneration of unknown pathogenesis was the only long-term abnormality noted. Major tissue iron deposits were confined to the liver, spleen, mesenteric lymph nodes and bone marrow, with only a slight sprinkling of hemosiderin in the pancreas and other organs. Although parenchymal iron deposition was seen, the predominant distribution was in the reticuloendothelial cells whether the iron came from hemoglobin catabolism or from saccharated iron oxide. No fibrosis or cirrhosis was produced by the iron deposits in the liver or elsehwere.

One of the principal problems in the pathogenesis of hemochromatosis is whether increased tissue iron deposits by themselves eventually produce cirrhosis and pancreatic fibrosis, or whether additional factors must be present. The results of these studies suggest that prolonged tissue iron-overload in concentrations comparable to those of human hemochromatosis is well tolerated, and in a 7-year period of observation does not of itself produce hemochromatosis in the dog.

Erythrocyte Radioiron Uptake in the Rat and the Effects of Cobalt Administration

By *Donald R. Korst and Frank H. Bethell.* Radioisotope Service, Ann Arbor V.A. Hospital, and the Department of Medicine, University of Michigan School of Medicine, Ann Arbor.

Twenty groups of 4 to 6 each of normal or hypophysectomized white (Sprague-Dawley) rats weighing 150–360 Gm. were given radioiron, and the response observed over a 3- to 5-day period. Several strengths of cobalt were given for 3 and 25 days prior

to the i.v. injection of Fe^{59}. Splenectomy did not alter the responses.

The methods used are patterned after those of Plzak et al. It has been found that minimal blood loss is important in this test, and that the best-suited rats are in the 150 to 200 Gm. weight range. Large rats have too high an uptake to be good test animals. The normal rats have an average % radioiron utilization, 1 day after injection, of 36 ± 10% (1 s.d.) the second day of 51 ± 9%, the third day of 48 ± 7% and the fifth day of 55 ± 11%. The fifth day determination was always on the uptake plateau, and therefore was eliminated. Hypophysectomized rats (10 days or more post-operatively) have the following average radioiron uptakes: first day, 12 ± 4%; second day, 19 ± 9%; third day, 32 ± 10% and fifth day, 28 ± 9%. The method entails i.v. injection of ferrous citrate (Fe^{59}) and withdrawal of .02-ml. blood specimens from which hemoglobin is determined and radioactively counted in 2-ml. aliquots. A standard to represent total injection radioactivity (about 2 μc.) is made up in the same syringe. Using these values plus the average weights (4.59% body weight, after Berlin et al.) and average hemoglobins of each rat, the following formula is applied:

$$\%\ \text{RBC } Fe^{59}\ \text{Uptake} = \frac{\text{c/m (2 ml. of sample)} \times \text{Wt. (Gm.)} \times \text{Hgb. (Gm./100 ml.)} \times .306}{\text{c/m (2 ml. of Std.)}}$$

The results are expressed as the average of each group of 4 rats.

Cobalt administration causes an increased radioiron utilization in the 70–100% range for normal rats, and in the 40–50% range for the hypophysectomized rat. The rate of red cell uptake is accelerated to a comparable degree whether the cobalt is given for 3 or 25 days. The effects of cysteine, histidine, and methylene blue on the effect of cobalt changes in ferrokinetics are being studied. Protein-free extracts of human plasma are also being tested using the rat radioiron uptake as an assay method.

Identification of Sickle Cell-Hemoglobin D Disease by Solubility Studies in Phosphate Buffer

By *Jerome L. Silverman and Amoz I. Chernoff.* Department of Internal Medicine, Washington University School of Medicine, St. Louis.

The identical electrophoretic mobility of hemoglobins S and D makes it impossible to differentiate the heterozygous combination, sickle cell-hemoglobin D disease, from homozygous hemoglobin S disease by electrophoretic technic alone. Furthermore, no method has been available to quantitate the percentages of hemoglobin S or D in a mixture of these 2 pigments. The discovery of a Negro female patient with homozygous hemoglobin D disease has made it possible to determine the solubility of reduced hemoglobin D in phosphate buffer using a slight modification of the method of Itano. In addition, a standardization curve has been constructed by using various combinations of hemoglobins S and D and plotting the percentage of each component against the solubility of the mixture.

The solubility of a specimen of reduced hemoglobin D was found to be 1.43 ± 0.01 (s.d.) Gm./L. in 2.58 M phosphate buffer at 25° C. Serial dilution of this specimen (90% hemoglobin D-10% S, 75% D-25% S, 50% D-50% S, 25% D-75% S, 10% D-90% S) was made with reduced hemoglobin solutions from 2 patients with homozygous hemoglobin S disease, the solubilities of which were 0.23 ± 0.01 Gm./L. and 0.30 ± 0.02 Gm./L. Both homozygous patients had approximately 2% hemoglobin F as shown by the alkali denaturation technic. Determination of the solubilities of the mixtures so prepared indicated a curvilinear relationship between the solubilities for 100% hemoglobin D and 100% hemoglobin S.

Since the solubility of a 50-50 mixture of D and S in both cases was more than twice that of S alone, this technic may be utilized to differentiate the 2 conditions, thus allowing further study of sickle cell-hemoglobin D disease. Also, use of the standardization curves allows the approximate percentages of D and S to be determined, once this disease is distinguished from homozygous S disease by the greater solubility of its hemoglobins.

Homozygous Hemoglobin D Disease

By *Amoz I. Chernoff, George Smith and Ruth Steinkamp.* Department of Internal Medicine, Washington University School of Medicine, St. Louis.

The prevalence of the heterozygous form of hemoglobin D (D trait) in the Negro has been established to be 0.4%. It may be calculated that the homozygous state for hemoglobin D should occur in 1 out of 250,000 Negroes. One such individual has been observed, and the syndrome of homozygous hemoglobin D disease has been characterized.

The patient, a 40-year-old Negro, had always been in good health except for some easy fatigability for 10 or more years. Physical examination was within normal limits. She has a light complexion and features are not typically negroid. Hematologic studies were as follows: red cells, 5,500,000 to 6,500,000 per $mm.^3$; hemoglobin, 12.5–13.5 Gm. %; hematocrit, 38%; MCV 67 μ^3; MCH, 23$\gamma\gamma$; MCHC, 35%. Leukocyte and platelet counts were normal; reticulocytes, 1.8%. The erythrocytes appeared somewhat hypochromic and microcytic with slight anisocytosis and poikilocytosis. Target cells comprised 60-70% of the red cells. The bone marrow revealed a moderate erythrocytic hyperplasia. The osmotic fragility curve was shifted symmetrically to the right with 10% hemolysis occurring at 0.34% saline and 90% hemolysis at 0.20% saline. Coombs' test was negative. Van den Bergh and liver

function tests were normal. Serum iron and iron-binding capacity were normal. The hemolytic index was 12. Cr^{51}-tagged red cells disappeared somewhat more rapidly than normal, half-time being 21 days. Paper electrophoresis studies of the patient's hemoglobin indicated a single component moving in the position of hemoglobin S. Sickling of the red cells was absent, and gelling could not be induced in a concentrated reduced solution of hemoglobin. Hemoglobin solubility in 2.58 M phosphate buffer at 25°C was 1.3 Gm./L. The alkali denaturation test for hemoglobin F was normal.

Family studies revealed that the patient's 3 sisters have hemoglobin D trait, while none of 3 half-brothers has hemoglobin D. Neither parent is living, but several of their relatives also lack hemoglobin D. In addition to Negro blood, white English and American Indian ancestry are present in the kinship. Although the genetic criteria for homozygosity for hemoglobin D cannot be proved, it seems likely that this patient represents an instance of homozygous hemoglobin D disease.

CIBHA Infuscusuria: A New Clinical Syndrome

By *Robert D. Lange and Joseph H. Akeroyd.* Department of Hematology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

A 12-year-old girl who presented a clinical syndrome of a hemolytic anemia with hitherto undescribed inclusion bodies in her erythrocytes has been studied at Walter Reed Army Institute of Research. The patient has been anemic since the age of 18 months and, in addition, has always passed dark brown to blackish colored urine. A splenectomy was performed at the age of 4, but transfusions were still required. From the age of 8 until recently she was maintained on cortisone.

The red cell inclusion bodies are refractile, irregular in shape and vary in size up to 2 μ. They are present in mature erythrocytes and reticulocytes but not in normoblasts of the bone marrow. The inclusion bodies have some of the characteristics of Heinz bodies, but are not identical. They are best demonstrated by use of the vital dyes. Electron microscopy reveals them to be relatively dense. The inclusion bodies examined spectroscopically demonstrated peaks of maximum absorption at 265 and 420 Å suggesting the presence of an abnormal hemoglobin or porphyrin-nucleoprotein complex. They are Feulgen negative.

Transfusion studies show a shortened survival of the patient's own cells and a normal survival of normal cells in the patient. The patient's cells disappeared rapidly when transfused into an infant with an intact spleen. These studies indicate the presence of an intracorpuscular defect of the red cells. Coombs' tests have been negative.

The urinary pigment is probably bilifuscin or a mesobilifuscin, but we have proposed the term infuscusuria until such time as the pigment is definitely identified.

This is believed to represent a new clinical syndrome: congenital inclusion body hemolytic anemia (CIBHA) associated with the passage of dark colored urine (infuscusuria). It is suggested that the disease may be due to an inborn error in nucleoprotein synthesis or maturation.

Nonaddisonian Megaloblastic Anemia

By *Norman A. Nelson and Ronald C. Bishop.* Department of Medicine, Wayne County General Hospital, Eloise, Michigan.

The recent introduction of radioactive vitamin B_{12} studies has provided a means for establishing the diagnosis of pernicious anemia in patients not presenting the classic hematologic findings. Pernicious anemia, either in relapse or remission, may now be diagnosed by demonstrating the absence of intrinsic factor activity by means of radioactive vitamin B_{12} studies. Conley has emphasized their value in patients with neurologic disease resembling subacute combined degeneration without anemia, or in those who have received inadequate therapy. Such studies may also be of help in excluding the diagnosis of pernicious anemia.

We have recently had the occasion to study 2 patients, both of whom had a macrocytic megaloblastic anemia with histamine-fast achlorhydria. One had a history of dietary insufficiency and purpuric skin lesions suggesting scurvy. He improved without definitive therapy other than diet. The Schilling test effectively excluded the diagnosis of pernicious anemia. The other patient was an epileptic on dilantin. It is interesting to note several recent reports from England on dilantin-induced megaloblastic anemia. An adequate hematologic response occurred in this patient during a trial with investigational material. The Schilling test, performed on 2 occasions, however, demonstrated normal urinary excretion of radioactive vitamin B_{12}, again eliminating the diagnosis of addisonian pernicious anemia and the need for continued vitamin B_{12} therapy.

In addition, a third patient with nutritional megaloblastic anemia was studied. Free hydrochloric acid was present in the gastric juice. A radioactive vitamin B_{12} excretion test was done, confirming the presence of intrinsic factor activity.

Cobalt^{60}-Labeled Vitamin B_{12} Absorption and Excretion Studies Using Timed Release Capsules

By *Philip C. Johnson and E. Stanley Berger.* Radioisotope Service V.A. Hospital, and the Department of Medicine, University of Oklahoma, Oklahoma City.

An attempt was made to investigate the site of vitamin B_{12} absorption in man by giving patients

without known neurologic or hematologic diseases an oral dose of 4 μg. of vitamin B_{12} labeled with 0.2 μc. of Co^{60}. The control group (*group 1*) was given the B_{12} as a water solution. A similar amount of vitamin B_{12} was given in spansules having 4 different release times. In *group 2* the spansules were designed to dissolve after entering the small intestine; in *group 3*, 1 hour release, in the upper small intestine; in *group 4*, 4½ hour release, in the middle small intestine; and in *group 5*, 7 hour release, in the lower small intestine. Each group contained at least 10 patients. Fecal excretion of the Co^{60} B_{12} showed: for *group 1*, 61.0 ± 6.01%; for *group 2*, 57.3 ± 4.76%; for *group 3*, 50.9 ± 4.30%; for *group 4*, 49.4 ± 5.11%; for *group 5*, 59.7 ± 4.23%. There is no statistically significant difference in these values (p greater than 0.10).

Simultaneous urinary clearance tests were performed on these patients. Two loading doses of 1.0 mg. each of vitamin B_{12} were given intramuscularly: the first simultaneously with the oral dose, the second, 24 hours later. The urinary excretion as % of the oral dose for the 0–24-hour collection period was: for *group 1*, 3.74 ± 0.65; for *group 2*, 4.62 ± 0.69 (p = 0.4), for *group 3*, 6.57 ± 1.29 (p = 0.1), for *group 4*, 6.16 ± 0.53 (p = 0.05), for *group 5*, 7.14 ± 1.03 (p = 0.02). For the 24–48-hour collection period it was: for *group 1*, 1.58 ± 0.01; for *group 2*, 4.17 ± 0.61 (p = 0.001); for *group 3*, 3.99 ± 1.34 (p = 0.2); for *group 4*, 5.15 ± 0.96 (p = 0.005); for *group 5*, 8.75 ± 1.99 (p = 0.005). In *groups 4* and *5* the urinary excretion during both collection periods was greater than those of the control *group 1*. These differences are statistically significant.

Based on the work of others, the previous binding by gastric juice, the site of absorption and the concentration of intrinsic factor are all thought to be related to vitamin B_{12} absorption. On the other hand, urinary clearance of vitamin B_{12} has been shown to be related to serum binding. Our data would suggest that the rate of release of vitamin B_{12} in the gastrointestinal tract influences both vitamin B_{12} absorption and urinary excretion. Furthermore, in the face of equal fecal excretion, the variable urinary clearance of vitamin B_{12} suggests that the site of absorption and the contact with gastric juice influences serum and liver binding of the vitamin.

Differentiation of Primary and Secondary Polycythemia by Pulmonary Function Studies

By *June M. Fisher, George N. Bedell and Paul M. Seebohm.* Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City.

The purpose of this paper is to present the findings of pulmonary function tests in patients with polycythemia—both primary and secondary—and to evaluate the place of these tests in studying such patients. Pulmonary function tests were done in 15 patients with polycythemia (hemoglobin 17.0 Gm. or greater and hematocrit 55% or greater). Measurements including lung volumes, maximal breathing capacity, maximal expiratory flow rate, evenness of alveolar ventilation, and arterial blood studies performed while the patient was breathing room air and after 100% oxygen breathing for at least 10 minutes.

Eight patients diagnosed as having polycythemia vera by hematologic criteria had essentially normal function studies. Arterial oxygen saturation exceeded 94% when the patients were breathing room air. One patient diagnosed polycythemia vera by hematologic criteria had abnormal pulmonary function tests and arterial oxygen saturation of 93% while breathing room air. He was found to have a pulmonary infarct. Six patients had significant abnormalities in pulmonary function and arterial oxygen saturation below 91% while breathing room air. The eventual clinical diagnoses in these patients were emphysema (3), pulmonary fibrosis (2) and pulmonary hypertension, cause not determined (1).

We conclude that if a patient has polycythemia and normal arterial oxygen saturation at rest, the diagnosis is polycythemia vera. Patients with polycythemia vera may have anoxemia at rest if they also have lung disease, venous to arterial shunt, medullary center depression or other cause for arterial anoxemia. Patients with secondary polycythemia do have anoxemia at rest. Pulmonary function tests are useful in the study of patients with polycythemia of unknown cause as they may reveal evidence to support a diagnosis of polycythemia vera, or an unsuspected cause for secondary polycythemia.

Mean Leukocyte Corpuscular Volume in Leukemia

By *William R. Best and Louis R. Limarzi.* Department of Medicine, University of Illinois College of Medicine, Chicago. (Aided by a grant from the U.S. P. H. S.)

Metabolic studies of leukocytes are often done in suspensions containing some erythrocytes. An approximate adjustment of data on the basis of gross protein weight may be made utilizing mean erythrocyte and leukocyte corpuscular volumes, relative numbers of the 2 cell types, and metabolic activity of erythrocytes alone.

Bloods were allowed to settle in Wintrobe hematocrit tubes for 1 hour and centrifuged at 3000 r.p.m. for 30 minutes. The buffy layer (BL) in per cent was noted. Simultaneous white blood counts (WBC) in cells/cm. were obtained. The mean leukocyte corpuscular volume (MLCV) in μ^3 was calculated.

$$MLCV = 10^7 \times BL/WBC$$

With WBC's less than 75,000 falsely high MLCV's were often obtained. Only bloods with WBC greater than 75,000 and with characteristic cells greater than 70% are analyzed below. Mean and standard devia-

tion of MLCV for various leukemias were: chronic lymphocytic (CLL, 29 cases), 246 ± 74; chronic granulocytic (CGL, 51 cases), 552 ± 140; acute granulocytic including acute exacerbation of chronic (AGL, 12 cases), 448 ± 119; and acute lymphocytic and unclassified (ALL, 10 cases), 260 ± 82. Except for ALL minus CLL, the differences between the various means are all statistically significant. No relationship could be established between MLCV and the differential count in CGL. Erythrocyte mean corpuscular volume in 360 leukemias of all types was 90.3 ± 9.5 μ^3.

These mean values have been helpful in correcting metabolic studies for erythrocyte contamination. In addition, these values permit a crude estimate of WBC when BL exceeds 4%. WBC is approximately 40,000 for each % of BL in ALL and CLL and, approximately 20,000 for each % in AGL and CGL.

Hodgkin's Disease of the Bone Marrow

By *William H. Bond and Robert J. Rohn.* Department of Medicine, Indiana University Medical Center, Indianapolis.

Hodgkin's disease, a malignant granulomatous disorder of the reticuloendothelial system, is frequently associated with nonspecific alterations in marrow constituents and, on autopsy examination, may show patchy invasion of the bone marrow cavity.

Because of the syncitial nature of the pathologic process, the attendant fibrosis or other unexplained causes, it is extremely rare to be able to demonstrate Reed-Sternberg cells on random needle aspiration of the marrow cavity.

Of the 80 patients with Hodgkin's disease seen in this clinic, 3 who had been diagnosed by standard fixed tissue technics, lymph node biopsy, etc., demonstrated numerous abnormal reticulum cells of the Reed-Sternberg type during the course of their disease.

The clinical features of these patients and their response to treatment are outlined. Photomicrographs of tissues prepared with hematoxylin and eosin and Wright's stain were made to determine the comparative cytologic characteristics of these cells, and time-lapse phase contrast microcinematographic studies were undertaken to show the motility behavior of these cells and, in one patient, the effect of CB-1348 upon the behavior of such cells.

A Coagulation Defect in Thrombocythemia Located in the Serum and Plasma

By *Irving A. Friedman, Arthur B. Dupee, Joseph H. Robbins and Frank C. Higgins.* Hektoen Institute for Medical Research, Cook County Hospital, Chicago.

Two cases of thrombocythemia associated with hemorrhagic diatheses, splenomagaly, leukocytosis, and marrow megakaryocytosis were studied. The coagulation profiles, including prothrombin consumption tests, were normal. However, the bleeding time was prolonged in both cases and thromboplastin generation was abnormal. The addition of normal serum or barium sulfate-treated plasma to the thromboplastin generation tests corrected this defect in 1 patient whose platelets behaved normally. The second patient showed partial correction of thromboplastin generation by either serum, barium sulfate-treated plasma or normal platelets. Therefore, both patients demonstrated a plasma and serum deficiency (simulating plasma thromboplastin antecedent [PTA] deficiency), and in 1 patient this defect was combined with a platelet thromboplastin deficiency.

The first patient developed marked thrombocytosis, bleeding and thrombotic episodes following splenectomy. After administration of P^{32} there was a simultaneous decrease in the number of platelets and bleeding time, with a disappearance of clinical symptoms. Repeat thromboplastin generation studies at this time were normal.

The common denominators of these 2 cases include prolonged bleeding times with normal prothrombin consumption tests. There was a definite correlation between the thrombocytosis, bleeding time and presence of a serum or plasma defect in 1 patient, which suggests association of the increased numbers of platelets with the serum-plasma defect.

Studies on the Heparin-Like Effect of Dextran Sulfate

By *Donald R. Griffith, Ricardo H. Landaburu, Josiah A. Polhemus, Park W. Willis, III and Ivan F. Duff.* Department of Internal Medicine, University of Michigan Medical School, Ann Arbor.

We have had occasion to investigate the anticoagulant effect of dextran sulfate, (Dexulate-Glaxo, London, England) a synthetic sulfated polysaccharide. The material (supplied in 5 cc. ampuls, 1000 U/cc.) was administered to a total of 12 patients. Eight had normal clotting mechanisms and received a single intravenous dose of 5000 U. Four patients who had thromboembolic disease (3 deep femoral thrombophlebitis and 1 probably pulmonary infarct) were given the drug in a constant intravenous infusion. Subsequent determinations were made of the Lee-White clotting time in all cases, the Quick 1-stage prothrombin time in 9 cases, the 2-stage prothrombin time (Ware-Seegers method) in 1 case, the modified Owren (Ware) prothrombin time in 2 cases, factor V (proaccelerin) in 3 cases, factor VII (proconvertin) in 1 case and fibrinogen in 1 case.

The clotting time was uniformly prolonged. This effect was marked (from 1½ to 10 times the control value) during the first hour after the single dose and persisted into the second hour on only 2

occasions. When the drug was administered by continuous infusion it was possible to control the clotting time by regulating the speed of infusion. The Quick 1-stage prothrombin time was promptly and significantly prolonged. This effect would last from 6–12 hours following the single dose. Factor V activity was markedly reduced in all 3 cases; this paralleled the effect on the Quick 1-stage prothrombin time. The modified Owren (Ware) prothrombin time, 2-stage prothrombin time, factor VII activity and fibrinogen levels were unaffected by the drug. No hemorrhagic or other toxic effects were noted in these short-term studies.

Some Observations on Hyperglobulinemic Purpura (Waldenstrom's Syndrome)

By *William E. Symon, Robert J. Rohn and William H. Bond.* Department of Medicine, Indiana University Medical Center, Indianapolis.

Abnormal capillary bleeding and chronic, recurrent purpuric states may be associated with, and the result of, previous formation of various abnormal serum proteins.

The abnormal serum proteins are globulins, and usually migrate with, or near, the γ globulin on electrophoretic migration. These abnormal globulins are roughly divided into 3 categories: (1) macroglobulins, (2) cryoglobulins and (3) hyperglobulins. All 3 may be associated with bleeding abnormalities.

Three cases have been studied manifesting hyperglobulinemic purpura. In 1, the disease is well advanced, demonstrating the classic clinical and laboratory alterations which have been outlined in the previous 18 published cases of this type. In the second, a shorter clinical course is associated with variability in clinical and laboratory manifestations, whereas the third is unique in being the first reported case of this disorder associated with, and possibly due to, a disseminated reticulum cell sarcoma.

Our investigations utilized hematological studies, hepatic function tests, clinical photographs, photomicrographs, representative free boundary electrophoretic and paper electrophoretic patterns and ultracentrifugal patterns.

S-Agammaglobulinemia and F-Gammaglobulinuria in an Unusal Myeloma Patient

By *Donald L. Howie, William Q. Wolfson and Joseph Weiner.* Department of Medicine and the Laboratory Service, U.S. Army Hospital, Fort Riley; and the Office of the Regimental Surgeon, Headquarters 18th Infantry Regiment, Fort Riley, Kansas.

A 49-year-old Negro woman first had pain in November 1954, diagnosed in May 1955 by clinical course, x-ray and marrow studies. P^{32} was followed by almost 1 year of complete symptomatic remission, including disappearance of azotemia. In May 1956 an acute relapse occurred with a peripheral blood and marrow picture of plasmablastic leukemia, with recurrence of renal failure and azotemia, and with hepatic impairment of urea synthesis (low blood urea/NPN, and, eventually, with sepsis which was fatal despite vigorous antibiotic, hormonal and supportive measures.

While the clinical course was consistent with myelomatosis, histopathology was not unequivocal: one opinion suggested acute monocytic leukemia. Serum electropapergrams showed elevated α_2 globulin (common and probably nonspecific in myeloma), markedly reduced β_2 globulin and absence of slow γ_2 globulin. Schiff staining was increased in all fractions except the fast γ globulin which also stained subnormally with toluidine blue, but not with mucicarmine. Both serum and urine were negative for cryoglobulin, pyroglobulin, macroglobulin and Bence-Jones protein. There was marked proteinuria without albuminuria. Its cause was an apparently homogeneous protein which migrated as a fast γ globulin and could not be even partially resolved from that of serum in mixtures.

Possible interpretations are: (1) only a partial agammaglobulinemia existed, or (2) there was complete agammaglobulinemia, and the fast γ globulin in serum actually was a myeloma component. Recent immunologic studies suggest the latter to be usually correct in apparent s-agammaglobulinemia. It seems probable in this patient because of an apparently identical urine protein with renal clearance greater than albumin, because of anomalous staining of the serum fast γ proteins, and because of the reduced β_2 globulin, which is largely immunoglobulin. The second view also clarifies the patient's refractoriness to antibiotic treatment.

Hemorrhagic Skin Lesions in Steroid-Treated Patients

By *Gunter M. Nashelsky, Charley J. Smyth, Glenn M. Clark and Kurt N. von Kaulla.* Department of Medicine, University of Colorado Medical Center, Denver.

This report concerns findings in a number of patients who have been on rather long-term steroid therapy. All have developed areas of painless subcutaneous hemorrhage, varying from petechiae to extensive ecchymoses. We have studied 10 patients (rheumatoid arthritis, 8; lupus erythematosus disseminatus, 1; intestinal lipodystrophy [Whipple's disease], 1). In each instance, "continuous" therapy with ACTH, cortisone, hydrocortisone or prednisone had been administered for not less than 3 years, and prednisone for not less than 13 months of this time. The most frequent site of involvement was the forearm and hand, occasionally the lower extremity—all areas subject to frequent trauma. In most instances, the skin lesions developed or became much more extensive during the period of prednisone therapy in doses ranging from 10 to 15 mg./day. No patient developed generalized symptoms.

It is considered most likely that these skin hemorrhages are the result of some effect upon the wall and/or supporting tissues of the blood vessels brought on by steroid treatment, and are not due to any abnormality in the coagulation system. Minor trauma appears to be a major contributing factor in these vascular lesions.

"Gelatinous" Bone Marrow

By *Norman A. Nelson and Leo P. Cawley.* Departments of Medicine and Pathology, Wayne County General Hospital, Eloise, Michigan.

In addition to the usual red and yellow bone marrow, a gelatinous form was recognized through its characteristic consistency by Bichat in 1812. It was believed to represent an abnormality occurring primarily in cachexia. Although this alteration of the marrow has been recognized for over 100 years, and in spite of considerable investigation into its nature by the early hematologists and pathologists, there is a paucity of related clinical studies.

Recent observations of 2 patients with grossly gelatinous marrow, noted upon aspiration, prompted further study. In both instances the appearance of marrow particle sections, stained with hematoxylin and eosin were identical. Ony scattered hematopoietic islands were present. The fat cells appeared small and were widely separated by large sheets of amorphous pink-staining material. Both patients suffered from severe normocytic anemia.

The first patient presented with the nephrotic syndrome and carcinoma of the prostate. The amorphous material in the marrow failed to give a positive reaction with the periodic acid-Schiff (PAS) reagent. The second patient was first seen with typical pernicious anemia in relapse, at which time the marrow was megaloblastic and of normal cellularity. In spite of continued vitamin B_{12} therapy, the patient returned with severe normocytic anemia, active pulmonary tuberculosis and cachexia. The amorphous intercellular marrow material in this instance gave a strongly positive PAS reaction. Serial studies have demonstrated improvement.

In summary, an accumulation of PAS-positive intercellular material normally present in minute amounts in the bone marrow may occur under certain circumstances. This should probably be differentiated from PAS-negative material which might represent simple edema fluid.

CARDIOVASCULAR SYSTEM

Primary Pulmonary Hypertension

By *Roy Behnke, James Carpentier and Dallas Fouts.* Department of Medicine, Indiana University School of Medicine, Indianapolis.

The principal physical findings in increased pulmonary blood flow are an accentuated pulmonary second sound, a diastolic left parasternal shock, a systolic and/or diastolic murmur, and right ventricular hypertrophy. When these are "paradoxically combined" with the roentgen features of scanty lung vasculature, which usually indicates diminished pulmonary blood flow, the diagnosis of primary pulmonary hypertension should be suspected.

The symptoms, although disabling, are paroxysmal and evanescent, so that the patient is often considered neurotic. Dyspnea of a severity disproportionate to the effort which produces it is a nearly invariable feature of the syndrome, but it may be accompanied by effort syncope and effort angina.

The three young adult women studied were symptomless at rest but incapacitated by dyspnea with slight exertion; 2 had effort syncope and 1 effort angina. Right heart catheterization revealed resting pulmonary artery pressures of 102/42, 72/34 and 70/27; the latter 2 pressures rose sharply to 91/42 and 106/49 with exercise. The highest resting cardiac output was 3.14 L./min., and the greatest exercise output was only 4.17 L./min. No left-to-right shunt was found, but in 1 patient the foramen ovale was traversed with the catheter. The left atrial pressure was −1.4 mm. Hg, while the right was −2.7 mm. Hg. That cyanosis can be produced through such a foramen was demonstrated during exercise, at which time the right atrial pressure rose to 3.5 mm. Hg, and there was prompt arterial oxygen undersaturation uncorrected by the administration of oxygen.

No adverse effects were noted during catheterization even with exercise, contrary to the experience of others. Diagnostic right ventricular hypertrophy was present on all electrocardiograms.

A necessary factor in the correct final diagnosis of the syndrome is the absence of lung disease. No evidence of such was found in these cases, and complete pulmonary function tests revealed no physiologic deviation from the normal.

Stenosis of a Branch of the Pulmonary Artery: A Cause for Continuous Murmurs over the Chest

By *Frederic L. Eldridge, Arthus Selzer and Herbert N. Hultgren.* Department of Medicine, Stanford University School of Medicine, San Francisco.

There have been rare reports of stenosis of a branch of the pulmonary artery, but no one has clearly pointed out its association with continuous murmurs heard over the chest.

Three patients, aged 5 to 7 years, all with other congenital cardiac malformations, have been shown by cardiac catheterization to have pressure gradients in a main branch of the pulmonary artery, indicating the presence of 1 or more stenoses of these branches. Two of the 3 patients exhibited continuous ductus-like murmurs heard at the chest wall over the site of the stenosis.

Both patients with continuous murmurs had pressure gradients across the area of stenosis throughout the cardiac cycle, whereas the patient with only a systolic murmur exhibited a pressure gradient only in systole. This finding indicates that the stenosis was the site of origin of the continuous murmur. Further proof has been provided by the experimental production of a continuous murmur in dogs in which partial constriction of a pulmonary artery branch was accomplished.

The finding of these stenoses in either right or left pulmonary arteries suggests that during cardiac catheterization pressure measurements should be made distally in both pulmonary arteries. This anomaly may not be as rare as the paucity of reports would indicate. Of real importance is the fact that these murmurs may mimic the murmur of patent ductus arteriosus.

Susceptibility of Dogs with Aortic Insufficiency to Endocarditis: Effect of the Hufnagel Valve

By *Joseph Roshe, Benjamin Highman and Paul D. Altland.* Clinic of Surgery, National Heart Institute, and the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.

Dogs with aortic insufficiency have proved highly susceptible to experimental endocarditis. Infections resembling acute and subacute bacterial endocarditis were produced consistently in 30 dogs by single and multiple intravenous injection of cultures of *Staphylococcus aureus* and *Streptococcus mitis* JH26, respectively. In addition to mitral and aortic vegetations, visceral infarcts and hemorrhagic and inflammatory lesions were seen in various organs. Dogs receiving *S. mitis* often developed, in addition, an acute diffuse glomerulonephritis, and occasionally focal glomerular lesions. No endocarditis was noted in 10 injected control dogs, including 2 with sham operations.

Hufnagel valves were inserted in 10 additional dogs with aortic insufficiency to determine if a reduction in cardiac work-load would reduce susceptibility to endocarditis. Three dogs were given *Staph. aureus* and seven *S. mitis*. Survivors were killed at 7-14 days. All dogs developed endocarditis. The severity of the endocarditis and other lesions was comparable to that seen in dogs without Hufnagel valves. The valve did not prevent diffuse and focal glomerulonephritis in dogs given *S. mitis*.

The data indicate that although the Hufnagel valve improves the altered hemodynamics of aortic insufficiency, it does not significantly lower the marked susceptibility of dogs with aortic insufficiency to endocarditis and glomerulonephritis. It is felt that the Hufnagel valve in patients does not lower their susceptibility to endocarditis.

Probable Mechanism of Ventricular Fibrillation and Cardiac Arrest Following Hypercapnia

By *Ananda S. Prasad, E. B. Brown, Jr. and Edmund F. Flink.* Medical Service, Minneapolis V. A. Hospital, Department of Internal Medicine and the Department of Physiology, University of Minnesota, Minneapolis.

Severe arrhythmias, including ventricular fibrillation, often occur during the first few minutes after return to air breathing following high CO_2 breathing. In previous work with dogs, plasma potassium concentration rose during high CO_2 breathing and increased further when the dogs returned to air breathing. However, the concentration of plasma potassium following 4 hours of high CO_2 breathing is not increased sufficiently to account for fibrillation or arrest by itself. The following experiments were carried out to determine changes in ultrafiltrable calcium and phosphorus.

Femoral artery blood samples were collected under oil from dogs anesthetized with sodium Pentothal. Several samples were obtained before, during 4 hours of 30% CO_2 breathing and during the recovery phase. Blood pressure and electrocardiogram were recorded simultaneously and continuously. The plasma was separated under oil. Ultrafiltrate of plasma was obtained by using a standard centrifuge, as previously reported. The ultrafiltrable calcium during respiratory acidosis increased slightly (0.4 to 0.6 mg./100 ml.) following 20 minutes of 30% CO_2 breathing. A marked decrease from the control concentration (1.6 to 3.3 mg./100 ml.) occurred at the end of 4 hours, and a further slight decrease occurred at the 5-minute recovery time. These changes occurred without significant changes in the total calcium. Both potassium and phosphorus increased.

The mechanism of cardiac arrest under these circumstances seems to depend upon the simultaneous reduction in plasma ionic calcium and the rise in plasma potassium concentration. A phenomenon similar to that exhibited by dogs has been noted in man by Miller et al. Certain unexplained postsurgical deaths may be due to rapid reduction in CO_2 tension, which had become elevated during anesthesia.

Lactic Dehydrogenase and Glutamic Oxalacetic Transaminase Levels in the Serum of Patients with Acute Myocardial Infarction

By *H. J. Zimmerman, C. G. Pilz and William Rock.* Department of Medicine, V. A. Hospital, Chicago.

The diagnostic value of serum transaminase levels in patients with myocardial infarction has been demonstrated by a number of recent studies. This highly sensitive index of myocardial infarctions, however, is helpful for only the first several days after the occurrence of the infarction, and almost always returns to normal by the 5th day.

In the present study of 25 patients with acute myocardial infarction, serum lactic dehydrogenase and transaminase levels were estimated serially. In all of these patients elevated levels of both enzymes were demonstrated. Lactic dehydrogenase levels, however, remained elevated for 6 to 12 days, while transaminase levels had returned to normal by 5 days or sooner. Patients with coronary pain, but without diagnosable infarction, had normal levels of transaminase and usually of lactic dehydrogenase. Patients in this category with congestive heart failure had mild lactic dehydrogenase elevations.

The degree of lactic dehydrogenase elevation was less marked than that of transaminase in patients with acute myocardial infarction, but there was a high degree of correlation between the relative degree of elevation of these enzyme levels.

Lactic dehydrogenase levels of the serum, while less specific and less sensitive than glutamic oxalacetic transaminase levels, remain clinically helpful for a longer period after an episode of myocardial infarction.

Mechanism of Increased Serum Activity of the Enzymes, Glutamic Oxalacetic Transaminase (SGO-T), Glutamic Pyruvic Transaminase (SGP-T) and Lactic Dehydrogenase (SLD), following Myocardial Infarction in the Dog

By *Irwin Nydick, Paul Ruegsegger, Felix Wroblewski and John S. LaDue.* Memorial Center for Cancer and Allied Diseases, New York City.

It has been previously demonstrated that increases in serum activity of the enzymes, glutamic oxalacetic transaminase (SGO-T), glutamic pyruvic transaminase (SGP-T) and lactic dehydrogenase (SLD) occur following myocardial infarction in the dog and man. The hypothesis was advanced that the mechanism of these rises was one of release of these enzymes into the blood stream from the necrotic myocardium. The present series of experiments was designed to study this hypothesis in more detail in man and in the dog.

That these increases are the result of leakage from necrotic myocardium is verified by the following findings: (1) The relative rises of serum enzyme activity are proportional to the original concentration gradients between the myocardium and the serum for each enzyme as well as to the size of the infarct. (2) The enzyme concentration in the infarcted muscle diminishes proportionally to the age of the infarct. (3) The most rapid rises in serum enzyme activity correspond to the period during which there is the most precipitous decrease in enzyme concentration in the infarcted myocardium. (4) Simultaneous samples of coronary sinus and peripheral venous blood show enzyme concentrations 10–15% richer in the samples from the coronary sinus. (5) In man, only 16 of 50 patients with coronary insufficiency and electrocardiographic abnormalities confined to the ST segments and T waves demonstrated any abnormalities in serum enzyme activity, whereas SGO-T and SLD increases are seen consistently following myocardial infarction. The low initial concentration of glutamic pyruvic transaminase in the heart muscle of man explains the irregular pattern of SGP-T variation following infarction. In the dog, experimental coronary insufficiency does not alter the serum enzyme patterns.

Progesterone and Alpha-Tocopherol in Experimental Epinephrine-Thyroxine Arteriosclerosis and in Cholesterol-Induced Atherosclerosis

By *Oscar F. Davis, Y. T. Oester and Bernard Friedman.* Department of Pharmacology and Experimental Therapeutics and the Graduate School, Stritch School of Medicine of Loyola University, Chicago.

A high incidence (89.5%) of a severe degenerative aortic sclerosis, essentially of the tunica media, is produced in rabbits by using epinephrine and thyroxine following the method previously reported by us. The lesions are rapidly induced in 15 days or less. Similarly, an essentially intimal atherosclerosis is induced in rabbits treated for 20 days or less with intravenous and subcutaneous injections of cholesterol suspensions. Alpha-tocopherol was studied in these scleroses because reports from Canadian and European sources have indicated that alpha-tocopherol produces improvement in clinical atherosclerosis. The reports of low β lipoprotein blood levels in young females, as well as low SF 10–30 Gofman fractions in pregnancy, led to the investigation of the effects of progesterone on these scleroses.

Five different groups of rabbits (8–19 per group) were given the epinephrine-thyroxine regime which produces the medial arteriosclerosis. In addition, each group received 1 of the following agents by subcutaneous injection: (1) progesterone, 50 mg./day; (2) progesterone, 75 mg./day; (3) alpha-tocopherol, 100 mg./day; (4) alpha-tocopherol, 200 mg./day; (5) alpha-tocopherol, 600 mg./day. None of these groups of animals demonstrated any signifi-

cant differences in incidence or severity of the induced medial sclerosis when compared to the control group of epinephrine-thyroxine alone.

Three different groups of rabbits (6–14 per group) were given the cholesterol regime which produces the intimal atherosclerosis. In addition, each group received 1 of the following agents by subcutaneous injection: (1) progesterone, 75 mg./day; (2) alpha-tocopherol, 200 mg./day; (3) alpha-tocopherol, 600 mg./day.

The group of animals receiving 75 mg./day of progesterone exhibited a much lower incidence of the intimal aortic sclerosis than the other 2 groups or the control group receiving only cholesterol. The incidence in the progesterone group was 46%, 6 animals in a total of 13 animals, while the control group had an incidence of 71.8%, 23 animals in a total of 32 animals.

Comparison of Reserpine and Deserpidine in Hypertensive Patients

By *A. S. Ridolfo, S. M. Chernish and B. L. Martz.* Lilly Laboratory for Clinical Research, General Hospital, Indianapolis.

Reserpine, Deserpidine (desmethoxyreserpine) and placebo were administered in a double blind study, each for a 6-weeks period in random order, to 11 patients with moderate primary hypertension. Dosage of reserpine and Deserpidine was 1 mg./day in 9, 0.5 mg./day in 2. Blood pressure and pulse were recorded in supine (after 5-minute rest) and standing positions at 2-week intervals.

Group averages of supine blood pressure readings were as follows: *lying*, placebo 182/112, reserpine 166/102, Deserpidine 169/103; *standing*, placebo 166/113, reserpine 155/107, Deserpidine 155/109.

Nasal congestion and dreams were reported more frequently during both drug periods than during placebo administration.

Cutaneous Necrosis due to Norepinephrine: Mechanism and Prevention

By *A. Stephen Close and Ross C. Kory.* Surgical and Investigative Medicine Service, V. A. Hospital, Wood, the Departments of Surgery and Medicine, Marquette University School of Medicine, Milwaukee, Wisconsin.

The increasing use of intravenous norepinephrine has been accompanied by frequent reports of cutaneous necrosis. The mechanisms involved in the production and prevention of this necrosis were investigated.

Leg veins were exposed in 7 dogs and norepinephrine was infused through polyethylene catheters. In 6 dogs, intense venospasm promptly appeared at the catheter tip. As the infusion continued, the spastic areas showed progressive and persistent blanching associated with disappearance of previously visible vasa vasorum. Tissue ischemia appeared to parallel the infusion vein rather than the tributary veins. Thrombosis did not occur cephalad to the catheter.

Four dogs received a standard series of subcutaneous norepinephrine injections into all 4 extremities. Slough resulted in 31% of these legs. In 16 dogs the incidence of slough was increased to 88% by inducing hemorrhagic hypotension prior to norepinephrine injections. In this series, 1 hindleg and 1 foreleg of each dog were utilized as controls (32 legs). The legs paired with the control legs received the same 4–6-hour exposure to norepinephrine, and were then infiltrated with either Regitine or Priscoline in saline solutions with or without hyaluronidase. Treated legs were not included in the results unless paired control legs developed sloughs. In 16 legs infiltrated with Regitine (12 in combination with hyaluronidase), no sloughs occurred. In 7 legs infiltrated with Priscoline (4 in combination with hyaluronidase), no sloughs occurred. In contrast, 5 legs were infiltrated with hyaluronidase alone; all 5 developed sloughs.

These observations strongly suggest that ischemia of the vein wall permits diffusion of norepinephrine into adjacent tissues. Increased sensitivity to norepinephrine induced by hypotension augments the vasoconstriction resulting from this diffusion. The resulting prolonged tissue anoxia leads to slough. Early local infiltration of adrenergic blocking agents is an effective means of preventing this complication.

ENDOCRINES AND METABOLISM

The Evaluation of Thyroid Function in Patients Receiving Carbutamide (Substance BZ-55)

By *G. W. Irwin, G. T. Lukemeyer, H. A. Soper and C. E. Test.* Department of Medicine, Indiana University Medical Center, Indianapolis.

Recently several new sulfonamides which cause hypoglycemia have been introduced and used in the treatment of diabetes mellitus. The possibility of side effects with long-term administration of these compounds has caused concern. It seemed important to determine if any antithyroid effect occurred with the administration of these sulfonamides. A few preliminary reports indicate depression of I^{131} thyroidal uptake following carbutamide.

In an attempt to compare any antithyroid ef-

fect of carbutamide with other known antithyroid drugs, I^{131} accumulation gradients were determined. A single dose of 2.5 Gm. of carbutamide caused no antithyroid effect such as that noted with methimazole.

Thyroid function was also studied before and at varying periods during carbutamide therapy. This report concerns a group of cases receiving 1 to 3 Gm. of carbutamide daily for 4 to 7 months. In this series there has been only slight fluctuation of the values for the BMR, PBI, 24-hour I^{131} thyroidal uptake and cholesterol determinations. In general, these tests have been within the normal range. There has been no thyroid enlargement or clinical features of hypothyroidism in these patients receiving carbutamide.

The Effect of Bromine Ion on I^{131} Thyroid Function Studies

By *Richard E. Peterson and Masa Yamamoto.* Radioisotope and Medical Services of the Iowa City V. A. Hospital, and the Department of Medicine, College of Medicine, State University of Iowa, Iowa City.

The observation that a clinically hyperthyroid patient had normal I^{131} tracer studies when first studied after 6 weeks of 3 Gm. daily of elixir triple bromides (ETB) aroused interest in the possibility of a bromine effect on radioiodine diagnostic studies.

Another hyperthyroid patient was studied with a tracer dose of Br^{82}. The thyroid concentrated 17–20% of the Br^{82} tracer dose and 55–70% of I^{131} tracer doses. At 24 hours, this patient had 57% of the serum I^{131} organically bound (butanol extractable), while an insignificant amount (less than 0.4%) of the serum Br^{82} was butanol extractable.

Twenty-five euthyroid patients were studied with I^{131} tracer studies before and on the third day of receiving ETB, 1 Gm. q.i.d. The PBI was not altered significantly. At 1 hour, the thyroid uptake of iodine was depressed by 2% of the dose ($p < 0.01$) and the 24th hour thyroid uptake was decreased 4.8% of the dose ($p < 0.1$). The 24-hour urine excretion of I^{131} was decreased 7% of the dose, with $p < 0.05$. I^{131} counts over the calf (body background) were increased following the ETB, averaging 35% increase at 6 hours and 18% increase at 24 hours. The total serum level of I^{131} and of butanol-extractable I^{131} were slightly, but insignificantly, decreased.

These data imply that the thyroid significantly concentrates but does not organically bind the bromine ion. Bromide, in the dosage given, depresses thyroid I^{131} uptake probably through competition in the halogen trapping mechanism. The decrease in urinary excretion of I^{131}, without change in the serum level of I^{131}, is compatible with the increased extrathyroidal I^{131} concentration indicated by the increased counts over the calf following bromide administration. The possibility of ETB administration interfering with I^{131} thyroid diagnostic studies must be kept in mind.

The Scintigram in Thyroid Disease Diagnosis

By *Robert E. Mack and Charles R. Carson.* Walter Reed Army Medical Center, Washington, D. C.

A study was made of the preoperative scintigram and the pathologic findings of 87 patients undergoing thyroidectomy. Interpretation of the scintigram was based upon a comparison of the scanning outline with the thyroid gland size as determined by physical examination. Any area with a reduction in the amount of normally functioning thyroid tissue appeared as a relative void in the radioactivity pattern.

The distribution of radioiodine was diffuse in all but one of 33 patients with adenomatous goiter. In an additional group of 33 patients having adenomatous goiter with hemorrhage or cyst formation, 26 exhibited a diminution of radioactivity in the area of disease. The finding of a normal scintigram in the remaining seven suggests that uniform involvement of the thyroid gland in the disease process may result in a parallel decrease in the iodine-concentrating ability. A similar explanation may be applicable to chronic thyroiditis, since 4 of 8 patients with this disease had normal scintigrams.

Of the 5 patients with papillary adenocarcinoma, 3 had normal scintigrams. The scanning abnormality in the other 2 appeared to be related to a coexisting colloid cyst rather than the carcinoma. Five of 8 patients with follicular adenoma or adenocarcinoma had apparently normal scintigrams. The failure of localization in these 5 patients may be related to the size of the lesion, since with one exception the diameter of the nodule did not exceed 1.5 cm. Radioautographs in 2 of these patients revealed a halo of functioning thyroid tissue surrounding the nodule.

The results of this study indicate that the value of the scintigram is limited by the sensitivity of the counter system for changes in distribution produced by small lesions, and the relative inaccuracy of visual estimates of radioiodine concentration. Serial scintigrams may be helpful in the evaluation of questionable changes.

Comparative Effects of Insulin and Orinase on Blood Levels of Pyruvate and Alpha-Ketoglutarate in Normal Subjects

By *Allen R. Hennes, Bernardo L. Wajchenberg, Stefan S. Fajans and Jerome W. Conn.* Metabolism Research Unit, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor.

In an effort to elucidate the mode of action of arylsulfonylurea compounds, a group of acute experiments have been performed on normal subjects to determine the effects of intravenous administration of insulin and of intravenous and oral administration of Orinase upon blood levels of glucose, pyruvate and alpha-ketoglutarate.

In 7 of 8 experiments an increase in the level of blood pyruvate was the earliest change associated with hypoglycemia produced by administration of insulin. In no instance did the level of pyruvate decrease before or during the time that blood sugar was falling. In contrast, following administration of Orinase, a decrease in the level of blood pyruvate was the earliest change associated with hypoglycemia in 7 of 9 experiments. This fall in the level of pyruvate preceded the fall in blood sugar in 3 subjects. In only 1 experiment was the earliest change in blood pyruvate an increase. There was no definite pattern of change in blood levels of alpha-ketoglutarate following intravenous administration of either insulin or Orinase.

Thus, the acute hypoglycemia following administration of insulin is usually associated with production of pyruvate in excess of its removal. Acute hypoglycemia following intravenous or oral administration of Orinase is usually associated with removal of pyruvate in excess of its production. These differences suggest that the immediate hypoglycemia induced by administration of insulin, on the one hand, and of Orinase, on the other, occurs via different mechanisms.

These results do not support a current concept that the sulfonylurea compounds produce acute hypoglycemia by stimulating rapid release of endogenous insulin.

Clinical Evaluation of Tolbutamide (1-butyl-3-p-tolylsulfonylurea) in the Treatment of Diabetes Mellitus

By *Robert H. Unger*. University of Texas Southwestern Medical School and the V. A. Hospital, Dallas.

A clinical evaluation of tolbutamide (1-butyl-3-p-tolylsulfonylurea) was undertaken in 20 diabetic patients who were classified into three groups: (1) severe labile diabetics, (2) moderately severe stable diabetics in whom insulin had been required for adequate control, (3) milder stable diabetics controllable with varying degrees of carbohydrate restriction without insulin.

Base-line studies were conducted for at least 7 days prior to beginning therapy. During this control period, and throughout the study, labile diabetics were maintained on suboptimal doses of insulin, while all stable diabetics were fed constant, calorically adequate, regular diets and received no insulin. After adequate base-line studies, tolbutamide therapy was begun with daily oral doses of 3 Gm. In unresponsive patients dosages were gradually raised to as high as 12 Gm./day.

Group 1 (3 patients): In no case was improvement observed during tolbutamide therapy. Profound insulin reactions occurred in 1 patient despite progressive deterioration in control of diabetes. Group 2 (8 patients): Decrease in fasting hyperglycemia and suppression of glycosuria was observed in only 3 patients. Group 3 (9 patients): Decrease in fasting hyperglycemia and suppression of glycosuria occurred in all 9 patients.

Untoward effects were encountered in 2 subjects. One patient developed urticaria, and generalized erythema occurred in another. In both, spontaneous clearing took place without termination of therapy. Liver function tests, urinalyses, complete blood counts and I^{131} uptake studies failed to reveal significant alterations.

The results confirm the effectiveness of tolbutamide in regulating many patients with mild and moderately severe stable diabetes, particularly the milder cases controllable with diet alone. Among the labile diabetics, not only was no benefit observed, but the concomitant administration of tolbutamide and insulin may possibly have been a factor in inducing hypoglycemic episodes.

Rapid Estimation of the Blood Glucose Concentration using Ordinary "Tes-Tape"

By *Holbrooke S. Seltzer*. Metabolic Service, V. A. Hospital, and the Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas.

Previous methods for the rapid estimation of the blood sugar concentration have not met widespread acceptance because they require special equipment and are cumbersome to operate. In contrast, the increasing utilization of Tes-Tape, the indicator paper containing glucose-oxidase which is used to detect urinary glucose, now makes available to everyone a fast and simple procedure for approximating the blood glucose level. The technic is based on the Tes-Tape color response either to undiluted plasma or to serial dilutions of plasma.

Whole blood glucose values (Somogyi) of 130 specimens of oxalated venous blood ranged from 16 to 760 mg. %. Corresponding plasma glucose levels were usually slightly higher, with a mean increase of 12 mg. %. The Tes-Tape response to plasma glucose always reflected the respective blood glucose level.

Simple determination of the Tes-Tape reaction to undiluted plasma enabled severe hypoglycemia or frank hyperglycemia to be distinguished from normoglycemia within 3 minutes after venipuncture.

More precise information, obtainable within another few minutes, was provided by the color responses of serial dilutions of plasma. The "end-point" was the weakest dilution of a plasma sample, showing a faint green tint on the yellow Tes-Tape one minute after application. Reading accuracy was proportional to the blood sugar concentration. At each plasma dilution representing an end-point, 90% or more of the corresponding blood glucose values fell within the parenthesized range: Plasma dilution (blood glucose): 0 (15–20 mg. %), 1:2 (20–40 mg. %), 1:4 (35–65 mg. %), 1:8 (45–95 mg. %), 1:16 (75–165 mg. %), 1:32 (145–355 mg. %), 1:64 (225–450 mg. %), 1:128 (500–760 mg. %).

The method described constitutes a valuable diagnostic aid and therapeutic guide in: (1) distinguishing hypoglycemic coma from other causes of unconsciousness, (2) evaluating the glycemic status of diabetic patients, and (3) following the blood glucose level during treatment of diabetic coma.

The Hyperglycemic Response as a Measure of Glucocorticoid Potency

By *Kelly M. West and James A. Hagans.* Department of Medicine, University of Oklahoma School of Medicine, and the V.A. Hospital, Oklahoma City.

The purpose of this report is to show that the hyperglycemic responses of human subjects are a reliable indication of glucocorticoid potency if a technic which includes glucose loading is used, and if the drugs tested have similar characteristics of time-action.

Twenty-one healthy ambulant young adults with no family history of diabetes were given 1.75 Gm./Kg. ideal body weight of glucose orally, 2 hours after which the venous blood glucose was determined. Subjects were given, on 5 subsequent occasions, the same simplified glucose tolerance test 2 hours after ingestion of cortisone acetate, prednisone, prednisolone, 9-α-fluorohydrocortisone and hydrocortisone.

Using a method of sequential analysis, only 3 cortisone-modified tests were required to establish the hyperglycemic effect of 50 mg. cortisone acetate after the results of the control tests of the group were known. Only 2 tests were required to establish the hyperglycemic effect of 10 mg. of prednisone, and 2 tests to establish the effect of 10 mg. prednisolone. Four tests established that 2 mg. 9-α-fluorohydrocortisone was ineffective, indicating by inference that it was less than 25 times as potent as cortisone acetate. The very subtle difference between the hyperglycemic potency of 40 mg. hydrocortisone and 50 mg. cortisone acetate was identified (hydrocortisone greater) after 12 tests by comparing the responses of the first 6 subjects tested. In each instance $p = < .01$. Forty-two tests in 21 subjects failed to identify any difference between the potency of prednisone and prednisolone.

The ability of a test requiring only 1 blood glucose determination to indicate glucocorticoid potency has been evaluated. This simple method yielded results which were in every instance comparable to those which have been previously established by more elaborate methods utilizing indices of "glucocorticoid" potency other than hyperglycemia. Furthermore, this type of testing seems capable of reliably identifying very small differences in potency.

Sodium Deficiency and Adrenocortical Hormone Secretion

By *Albert B. Eisenstein and Phyllis Hartroft.* Departments of Preventive Medicine and Pathology, Washington University School of Medicine, St. Louis.

Although sodium deprivation evokes hypertrophy of the zona glomerulosa and increased aldosterone secretion, it is not known whether production of other adrenocortical hormones is altered. The effects of sustained sodium restriction on adrenal steroid secretion have, therefore, been investigated.

Young male Wistar rats were placed on a synthetic diet deficient in sodium. Pair-fed controls received the same diet to which 0.6% sodium chloride was added. Animals from both groups were killed 2, 4, 7, 9, 14, 30 and 60 days after beginning the diets, and serum sodium, potassium, blood hematocrit and adrenal weights were determined. One adrenal from each rat was incubated aerobically at 37°C. in a buffered medium containing ACTH. After 2 hours incubation, steroids secreted were recovered and quantitatively determined by a spectrophotometric technic. This procedure measures all biologically active steroids secreted by the rat adrenal, and thus represents total hormone production of this gland. In addition, the secretion of individual adrenocortical hormones was determined by paper chromatographic analysis. The remaining adrenal from each rat was used for histologic study.

The results demonstrate that total steroid secretion by the adrenals of control rats increased progressively as the animals grew. Total adrenal steroid production by sodium-deficient animals remained constant throughout the experiment and was significantly ($p = < 0.01$) lower than in controls.

Chromatograms revealed increased secretion of aldosterone by the adrenals of sodium depleted rats, but synthesis of corticosterone and other hormones was reduced. These changes were correlated with diminished serum sodium concentration and increased size of the zona glomerulosa.

It is concluded from these observations that prolonged sodium depletion results in increased synthesis of aldosterone, but the secretion of other adrenocortical hormones is greatly diminished. It is suggested that these changes may interfere with the ability of the animals to withstand stress of other types.

Importance of Anemia in Artificially-Induced Metabolic Acidosis

By *Edwin G. Olmstead, Donald A. Roth and John H. Lunseth.* Departments of Medicine and Pathology, Milwaukee County General Hospital and Marquette University School of Medicine.

Three patients with severe blood loss anemia were studied in an attempt to assay the importance

of red blood cell and hemoglobin buffers in overcoming metabolic acidosis. After initial blood pH, nonprotein nitrogen, bicarbonate, chloride, sodium and potassium were obtained, these patients were given 50 mEq. of ammonium chloride intravenously per hour for a 4-hour period. At hourly intervals a blood pH, blood bicarbonate, urinary pH, and total titratable acidity were determined. Two hours after the end of the infusion, 233 mEq. of sodium bicarbonate were administered intravenously, and blood and urinary studies were continued as above.

Following this, patients were transfused with red cell mass to normal hemoglobin levels and red blood counts, and the procedure was again carried out exactly as above.

The study showed no significant change in range of blood pH, bicarbonate, urinary pH or titratable acidity during and following ammonium chloride infusion in this period of anemia, as compared with a period of normal red blood count and hemoglobin levels. No difference in response to infusion of sodium bicarbonate during the state of compensated metabolic acidosis was noted when patients were anemic, as compared with when they had a normal red blood count and hemoglobin level. It was concluded that correction of severe anemia is not an important factor in overcoming ammonium chloride-induced metabolic acidosis nor in correction of this acidosis with sodium bicarbonate.

The Effect of the Administration of an Intravenous Fat Emulsion upon the Blood Lipids of Normal and Hospitalized Subjects

By *Jack M. Iacono, William W. Cleland, Lucille Palm and John F. Mueller.* Army Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver.

A study was made of the effect of an intravenous infusion of a fat emulsion on the various serum lipid fractions in 9 healthy volunteer laboratory workers and 9 patients selected at random from a surgical ward of a general hospital. Blood samples were obtained before, at the end and 2 and 4 hours after the end of a single infusion of 600 ml. of a 15% cotton-seed oil emulsion. The whole serum and its subnatant, recovered after centrifugation at 28,000 g for 1 hour, were analyzed for total fatty acid, free and total cholesterol, phospholipid and unesterified fatty acid. It is a reasonable assumption that the subnatant contains most of the soluble lipoprotein, and the supernatant the chylomicron fat.

The concentrations of various lipid components were highest at the end of the infusion, and gradually declined toward normal thereafter. Although there was some individual variation, the hospitalized subjects showed lower concentrations of all fractions than the volunteer subjects, indicating more rapid "clearing" of the infused fat by the former. The concentrations in the whole serum and subnatant paralleled one another in the phospholipids, while minor variations occurred in total fatty acids. The most interesting variation occurred in cholesterol, wherein the values in the whole serum followed the expected rise and fall but the subnatant value progressively diminished.

The results of these studies suggest that the transfer of cholesterol from soluble lipoprotein to the chylomicron fraction may be important in fat transport. Cholesterol may function as an actual fat carrier or as a simple emulsifier.

Metabolic Interrelations of Calcium and Magnesium in Patients With and Without Osteolytic Disease

By *William O. Smith and Leonard P. Eliel.* Oklahoma Medical Research Foundation and V.A. Hospital, Oklahoma City.

Hypomagnesemia of 0.9 to 1.1 mEq./L. with convulsions occurred in 3 patients with osteolytic disease in association with episodes of hypercalciuria, hypercalcemia and renal impairment. Urine excretion of calcium and magnesium was followed daily for a prolonged period. Urine magnesium was found to vary directly with urine calcium, and in a greater amount than could be accounted for on the basis of bone destruction alone; when urine calcium increased by 100 mg. daily, magnesium increased by 6 mEq. daily. When a hypercalciuria of kelated calcium was produced in 1 of the patients with intravenous EDTA, however, no increase occurred in urinary magnesium.

Two patients without osteolytic disease and with normal renal function were studied with daily intravenous calcium loading while on metabolic balance. Here an inverse relationship was noted: the urine magnesium decreased to 25% of the control values, while urine calcium increased by 700%. However, the serum magnesium declined from 1.9 mEq./L. to 0.8 mEq./L. in 1 patient and from 1.6 mEq./L. to 1.2 mEq./L. in the other. Stool magnesium showed no change. A diuresis of sodium, chloride and water occurred in both patients. It is postulated that the magnesium moved intracellularly. Neither of these patients developed symptoms of hypomagnesemia. No changes in serum or urine magnesium were seen with infusions of an equivalent amount of sodium.

It appears that the normal metabolic response to intravenous calcium loading is an intracellular shift of magnesium with a resultant decrease in extracellular concentration and in renal excretion of this ion. Patients with osteolytic disease show increased renal loss and extracellular depletion of magnesium during hypercalciuria of ionized calcium, possibly due to tubular damage from the intermittent hypercalcemia occurring in such patients.

5-Nucleotidase Activity of Human Serum

By *Irving I. Young.* Department of Medicine, Wayne State University College of Medicine, and City of Detroit Receiving Hospital, Detroit.

5-Nucleotidase, a phosphatase specific for nucleotides containing a phosphate esterified at carbon 5 of the pentose, is widely distributed in human tissues. In serum, this enzyme has at pH optimum of 7.5 to 8.0 and is strongly activated by magnesium ion.

Quantitation of 5-nucleotidase activity in serum by determining the rate of hydrolysis of 5-nucleotides is unsatisfactory because of the presence of nonspecific alkaline phosphatase. Selective inhibition of alkaline phosphatase is possible by preincubation of serum with 0.0015 M ethylenediaminetraacetic acid. At this concentration of ethylenediaminetetraacetic acid 5-nucleotidase is either unaffected or somewhat activated. A unit of 5-nucleotidase activity has been established as equivalent to the liberation of 1 mg. of phosphorus as the phosphate ion during 1 hour of incubation at 37° and pH 7.5 with a substrate containing adenosine-5-phosphate and magnesium ion.

Normal values for adults range from 0.3 to 3.0 U/100 ml. serum. Significant elevations are observed in the presence of biliary tract obstruction, frequently but not invariably parallel to serum alkaline phosphatase activity.

In agreement with the findings of Dixon and Purdom, serum 5-nucleotidase activity is not elevated in diseases of bone associated with high alkaline phosphatase values.

The Effect of Ether Anesthesia on Pyruvate and Lactate Metabolism

By *William R. Drucker, Christine Costley, Robert Stults, William Holden, Max Miller, James Craig and Hiram Woodward.* Departments of Surgery and Medicine, University Hospitals and Highland View Hospital, Cleveland, Ohio.

Inhibition of carbohydrate metabolism in the cell may be responsible for ether narcosis. This possibility has been extensively investigated in tissue slices, but relatively few studies have been conducted in man. Our previous studies in man revealed that ether anesthesia caused a marked decrease in glucose tolerance but relatively little change in fructose tolerance. This was interpreted as indicating that ether inhibits specific enzymatic processes.

The present study was designed to determine the effect of ether on pyruvate and lactate metabolism, since pyruvate is the final step in the anaerobic scheme of glycolysis, and it supplies, via acetyl CoA, a main source of fuel for the citric acid cycle.

Ten volunteers were given 2 pyruvate or lactate tolerance tests, 1 as a control and 1 during ether anesthesia. The sodium pyruvate (0.12 Gm./Kg.) or sodium lactate (0.11 Gm./Kg.) was given intravenously for 1 hour by a constant infusion pump. Arterial blood oxygen was followed by an oximeter and serial measurements of blood gases. The results revealed a marked disturbance in tolerance for both pyruvate and lactate during ether anesthesia despite adequate arterial oxygenation. The average rise during the initial 30 minutes of infusion was: 1.33 mg.% (control) and 3.13 mg.% (anesthesia) for pyruvate; and 8.8 mg.% (control) and 21.4 mg.% (anesthesia) for lactate. The results are of interest in regard to the possibility that decreased energy production due to impaired pyruvate and lactate metabolism may result in narcosis.

Effects of Pyrazinamide on Serum and Urine Uric Acid

By *Donald F. Gleason, John P. Street and Kenneth A. Kahn.* Department of Medicine, V.A. Hospital, Minneapolis.

Patients being treated with pyrazinamide (3 Gm./day) for tuberculosis were found to exhibit hyperuricemia. Serial 24-hour urine and serum urates were measured in patients and controls, using a uricase ultraviolet spectrophotometric method. Pyrazinamide itself did not interfere with this method.

In 6 male patients, serum urate ranged from 9.4 to 13.6 mg.% (10 controls, 3.6 to 6.1 mg.%) and urine excretion ranged from 140 to 395 mg./day (ten controls, 400 to 740 mg./day) on ordinary hospital diet. Serum urate was 8.2 mg.% and excretion 220 mg./day in 1 female patient.

Pyrazinamide was administered to 3 males after control periods. Urinary excretion decreased precipitously to extremely low levels and then rose slowly into the above subnormal ranges. Serum urate rose rapidly and remained elevated. Simultaneous creatinine determinations and other clinical renal function tests revealed no abnormality, except that PSP excretion ability was depressed in some patients. On discontinuing pyrazinamide, the urate abnormalities reversed promptly, but an excess of urates was excreted for about 1 week.

One patient with no previous gout developed acute gouty arthritis shortly after pyrazinamide was discontinued. However, his serum urate remained elevated. Pretreatment levels were not available. It appears probable that he had asymptomatic gout and the induced abnormalities simply precipitated his first attack.

Increased tubular reabsorption of urates appears to be involved, but alternatives, such as altered ultrafiltrability of urates, are not excluded. Decreased urate excretion persisted after many months of pyrazinamide therapy, apparently reflecting decreased production. This may be a physiologic response to elevated serum levels, but may represent more fundamental disturbance in urate metabolism. The observed phenomena present potential tools for further study of uric acid metabolism and gout.

GASTROINTESTINAL SYSTEM

Significance of Increased Urinary Pepsinogen (Uropepsin) Excretion in Duodenal Ulcer and During ACTH Administration

By *B. I. Hirschowitz, D. H. Streeten and H. M. Pollard.* University Hospital, Ann Arbor, Michigan.

To determine whether the mechanisms of increased uropepsin excretion due to ACTH were the same as those responsible for the increases in patients with duodenal ulcer, pepsinogen clearances were measured simultaneously with gastric pepsin secretion in 5 normal males before and during 6 days of ACTH-gel administration, as well as in 6 ulcer subjects with high uropepsin excretion.

In the normal controls and in the ulcer subjects good linear correlation ($r = +.92$) existed between urinary and plasma pepsinogen. The mean pepsinogen clearance in ulcer patients (36 ± 19 L./24 hr.) did not differ significantly from that in normals (44 ± 14 L./24 hr). ACTH administration, however, invariably produced a steep sustained rise in urinary pepsinogen excretion without change in plasma pepsinogen, the mean clearance increasing to 65 ± 29 L./24 hours. Thus, the increased uropepsin excretion in ulcers resulted from elevated blood levels of pepsinogen with a normal renal clearance, whereas the increased uropepsin excretion during ACTH occurred with a fixed plasma pepsinogen and was due to an increased renal clearance of pepsinogen.

The differences between pepsinogen clearances and GFR probably imply some glomerular impediment to excretion, as well as some tubular resorption of pepsinogen (Mol. Wt. 44,000), as is the case with albumin (Mol. Wt. 47,000). The positive correlation between endogenous creatinine and pepsinogen clearances in the normals ($r = +.68$), and increases in both by ACTH ($p < .01$), though proportionately different, (13 and 48%, respectively), suggested that the increases of uropepsin by ACTH may partly be due to increased glomerular filtration of pepsinogen. The uropepsinuric effect of phloridzin (Peczenik) suggests, however, that tubular resorption plays some part in regulating uropepsin excretion.

The relation between gastric pepsin secretion and uropepsin excretion is erratic—ratios vary from 100:1 to 3000:1—even in the same subject, and various substances may affect one independently of the other. This is not surprising, since at least 3 factors may be responsible for elevation of uropepsin excretion: (1) increased peptic cell mass (e.g., long-standing duodenal ulcer), (2) reversal of secretion gradient of peptic cells resulting in elevation of plasma pepsinogen, and (3) increased renal clearance of pepsinogen as produced by ACTH.

The Effect of Serotonin and Lysergic Acid Diethylamide on the Secretory Response to Reserpine

By *James B. Hammond.* Lilly Laboratory for Clinical Research, Indianapolis General Hospital, Indianapolis.

Because Shore, Silver and Brodie have demonstrated an inhibition of serotonin- and reserpine-potentiated effects of certain hypnotics by lysergic acid diethylamide, it seemed of interest to study the effects of these agents on gastric secretion.

In dogs receiving serotonin intravenously in doses which stimulated intestinal motility, no gastric secretion ensued. Likewise, there was no gastric secretion following the intravenous injection of LSD at a dose producing generalized muscular rigidity. As has been previously demonstrated, the injection of reserpine was followed by a consistent and pronounced gastric secretion. This response was not affected by the simultaneous injection of serotonin but was reduced by the administration of LSD.

These data suggest that peripheral as well as central actions of reserpine may be inhibited by LSD, but not by serotonin.

The Use of Alcohol as a Liver Function Test

By *David G. Pietz, B. D. Rosenak and R. N. Harger.* Gastrointestinal Clinic, Indianapolis General Hospital, Indianapolis.

Ninety-five % ethyl alcohol, diluted to 25%, was ingested in a dosage of 0.5 Gm./Kg. by 75 subjects as a test for liver function. This is patterned after tests devised by European workers who found that cirrhotics had a higher 4-hour alcohol blood level than normals. Similarly, 3- and 4-hour determinations (the former as a check of the procedure) were obtained using instead, however, a modified Harger Drunkometer rather than blood analysis per se. Four-hour levels of 0–10 mg.%, 10.1–20, and 20.1+ were considered normal, borderline and abnormal respectively. Modifications of the Drunkometer procedure include using the rebreathing technic outlined by Harger, an incubator for maintenance of breath sample at 28°C., 0.25 ml. of K_2MnO_4 in the reagent, and an alteration of the color standards. One standard employs 2 ml. of 5% $CoSO_4$ with 13 ml. of water. The other uses 1 ml. of 5% $CoSO_4$, 1 ml. of 0.1% $K_2Cr_2O_7$ and 18 ml. of water.

The alcohol test was compared to the 45-min. bromsulfalein determination, which in turn has been classified into normal, borderline and abnormal categories. Each was subdivided into presence or absence of corroborative evidence of liver disease. The latter included clinical history and findings, cephalin flocculation, thymol turbidity, bilirubin and alkaline phosphatase tests.

In the normal bromsulfalein range (37 subjects) there were 2 (5.4%) unexpected results. The inci-

dence of borderline alcohol levels was high when there was other evidence for liver disease. In the borderline bromsulfalein category (5.1–10%), no discrepancies were found. In the abnormal bromsulfalein range (25 patients), the unexpected results were 28%, though they may be as high as 40%.

The alcohol test is most useful in diagnosing mild liver disease in contrast to determining prognosis and differential diagnosis of jaundice. An abnormal value appears to be highly significant.

Hepatic Function and Morphology in Chlorpromazine Jaundice as Affected by Continued Administration of Chlorpromazine

By *Edward M. Schneider, Charles Daugherty and James K. DeVore.* Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Four patients who developed jaundice while receiving chlorpromazine were studied throughout the course of jaundice while administration of the offending agent was continued in unchanged dosage.

In all 4 patients a group of laboratory tests including liver function studies, serum electrophoresis and lipid phosphorus determinations were done on at least 3 occasions during the periods of observation. In three of the 4 subjects a liver biopsy specimen was obtained at the time of onset of icterus, and again after all clinical and laboratory evidence of jaundice had cleared.

Elevation of serum bilirubin and alkaline phosphatase was noted in every subject at the time of onset of clinical jaundice. In all patients the initial lipid phosphorus was greater than the final determination at completion of the study. The total cholesterol levels paralleled these findings in every patient. In 2 subjects the first liver biopsy revealed periportal infiltration and bile canalicular plugging. The biopsy on the third subject demonstrated only slight vacuolar fatty change. Repeat biopsies (63–78 days later) in all 3 patients were normal except for persistent slight fatty change in 1.

In three of the four subjects all evidence of jaundice disappeared within 10–21 days. In the fourth subject normal serum bilirubin levels were not obtained until 78 days after onset of jaundice.

The complete return to normal of hepatic function and morphology while chlorpromazine administration was continued effectively dispels the notion that this drug is a specific hepatotoxin. However, the findings herein reported could well find their etiology in one of two other theoretic mechanisms: (1) a hypersensitivity to chlorpromazine, or (2) a combination of biliary dysergia and water diuresis (with resulting dehydration) leading to bile stasis and canalicular plugging.

The Effect of Oral Ethanol on the Hepatic Glutamic Pyruvic and Glutamic Oxalacetic Transaminase in the Rat

By *Keith S. Henley, Hugh S. Wiggins and H. M. Pollard.* Department of Medicine, University Hospital, University of Michigan, Ann Arbor.

The effect of alcohol was tested on 4 groups of rats: (1) those receiving 20% alcohol as the sole source of fluid, and on an ad lib diet (10 rats); (2) those receiving water, and pair-fed with those from group 1 (10 rats); (3) those receiving water, pair-fed with group 1, and isocaloric by means of fat supplements (10 rats); (4) those receiving water and fed ad lib (12 rats).

The diet was adequate with respect to lipotropic factors and vitamins, which also were present in the drinking fluid. One-half the animals were killed after 8 weeks, and the remainder after a further 17 days of halved food intake and consequent weight loss. Glutamic pyruvic (GPT) and glutamic oxalacetic (GOT) transaminase determinations were carried out in duplicate on the livers and brains.

After 8 weeks, the mean hepatic GPT activity was significantly lower in group 1 than in group 3 in terms of dry weight, and than group 4 in terms of dry weight and nitrogens. This difference was enhanced by starvation, becoming significant between groups 1 and 2 also. It was significant between group 1 and any of the other 3 groups in the starved and nonstarved animals taken together, in terms both of dry weights and nitrogens. Upon starvation, there was also a significant increase in hepatic GOT activity in groups 1 and 2.

These changes were not reflected in the transaminase determinations on the brains. As the nitrogen contents of the livers were similar in the various groups, the differences were independent of the extent of any fatty changes.

The results show that alcohol causes a reduction in hepatic GPT activity that is enhanced by starvation and is indicative of a direct action of alcohol on the liver.

Percutaneous Transhepatic Cholangiography

By *William T. Fitzgerald, James C. Redington and William A. Knight, Jr.* Department of Internal Medicine, St. Louis University School of Medicine, St. Louis.

A method of cholangiography by direct instillation of radiopaque dye into the biliary system has been investigated. Results obtained in the study of 14 patients revealed the following lesions of the biliary system: 2 benign obstructions, 11 malignant obstructions and 1 case unsatisfactory for interpretation.

The procedure was performed under local anes-

thesia, following Demerol and atropine premedication. In all cases a transthoracic approach was utilized. The site selected was between the anterior axillary and midclavicular lines, one or two intercostal spaces below the superior border of liver dullness. A 6-inch, 20 gage needle was inserted at this site and directed toward the hilum of the liver. Continuous suction was maintained during insertion of the needle. When a major biliary duct or radicle was encountered, 20 to 40 cc. of the hepatic bile was aspirated and saved for analysis. Twenty to 30 cc. of 50% Hypaque was then injected into the biliary system, following which appropriate x-rays were made.

With this technic we obtained cholangiograms in 13 of the 14 cases. The findings demonstrated obstruction of the biliary system ranging in location from the ampulla of Vater to the porta hepatis. We encountered bile leakage in 2 cases but no fatalities.

INFECTIOUS DISEASES

Penicillinase Formation and Penicillin Resistance in Neomycin-Resistant Staphylococci

By *Paul R. Finley and Wendell H. Hall.* Laboratory Service, V. A. Hospital, Minneapolis.

While testing the effect in vitro of combinations of neomycin and penicillin against strains of staphylococcus, a phenomenon was observed which has not been reported heretofore. Two strains of hemolytic, coagulase-positive *Staphylococcus aureus* (#9 and #14), obtained from the blood of patients, were exposed separately to neomycin and penicillin. Both strains were highly susceptible to both antibiotics as determined by tube-dilution and disc technics prior to exposure.

A moderate degree of resistance to the drugs was established by serially transferring the organisms into progressively higher concentrations of the antibiotics. It was observed that the two strains which were rendered resistant to penicillin alone (#P9 and #P14) were effectively inhibited by neomycin. On the other hand, the same two strains exposed to neomycin alone (#N9 and #N14), showed definite resistance to both penicillin and neomycin, especially with a large inoculum. This fact led to the suspicion that the 2 strains (#N9 and #N14) exposed solely to neomycin were able to fabricate a substance capable of negating the effect of penicillin on the staphylococcus in vitro.

From the 2 strains of *Staphylococcus aureus* (#N9 and #N14) that had been exposed to increasing amounts of neomycin, an extract containing a penicillin-inhibiting substance was prepared according to the method of Harper. The extract was able to nullify the bactericidal action of penicillin on a susceptible staphylococcus to a significant degree. The larger the amount of extract added, the more effective was the inhibition. The 2 strains (#N9 and #N14) grown in neomycin alone retained the ability to produce the inhibiting substance after repeated subculture on antibiotic-free media.

The 2 strains of staphylococcus (#P9 and #P14) grown solely with increasing amounts of penicillin, did not produce an inhibitor to penicillin, albeit they became somewhat more resistant to penicillin than the parent strains, #9 and #14. Large populations of the parent strains contained no demonstrable penicillin-resistant variants nor penicillinase producers.

Strains of staphylococcus in which penicillin resistance has been induced by repeated exposures to penicillin in vitro seldom produce penicillinase, and the resistance is only temporary. Neomycin therapy seldom leads to resistance in the staphylococcus to either neomycin or penicillin, but the present experiments suggest that individuals treated with neomycin might harbor staphylococci resistant to penicillin even though there has been no administration of penicillin. The mechanism of cross-resistance to neomycin and penicillin may be on the basis of the ability of neomycin to induce penicillinase production in the staphylococcus.

Long-Term Follow-up of Patients with Healed Bacterial Endocarditis

By *Buford Hall.* Department of Medicine, University of Illinois College of Medicine, Chicago

This study was undertaken to determine the long-term prognosis of healed bacterial endocarditis and to note factors which influence the prognosis. The present status of all 17 patients who were discharged from the hospital cured of bacterial endocarditis 6 or more years ago was determined. During the first year after antibiotic treatment, 5 patients died—3 of congestive heart failure and 2 of noncardiac causes. Two additional patients died 26 months and 6 years after penicillin therapy, of coronary heart disease and congestive failure, respectively.

Of 10 living patients, followed 6 to 10 years, 4 are in Class I (functional capacity, A.H.A.), 5 are in Class II, and 1 is in Class III. The patient followed for the longest time has had 2 children and no cardiac symptoms whatever.

The total dosage of penicillin administered, the duration of therapy and the presence of previous treatment had no demonstrable effect on the prognosis in these patients.

The mean ages at the time of the infection of the 10 survivors and of the 5 who died of heart disease were 31.5 and 40.8 years, respectively. Four of the latter group had aortic regurgitation (3 with cardiomegaly), while only 3 of the 10 survivors had aortic regurgitation (1 with cardiomegaly) at the time of hospitalization. Of 7 patients with aortic regurgitation, 4 are now dead and the other 3 have shown a decrease in functional capacity. Of 9 patients having only mitral valve disease, 2 are dead (1 of tuberculosis), 3 have worsened symptomatically, and 4 are unchanged. A patient with the tetralogy of Fallot died following thoracotomy.

This study shows that some patients may remain well 6 to 10 years after recovery from bacterial endocarditis, especially if aortic regurgitation is not present after therapy.

The Neutralizing Antibody Response to Adenovirus Infection

By *J. Thomas Grayston, Clayton G. Loosli, Paul B. Johnston, Mabel E. Smith and Robert L. Woolridge.* University of Chicago School of Medicine, Chicago; the Naval Medical Research Unit No. 4, USNTC, Great Lakes, Illinois.

Studies were performed on 53 paired serums from patients suffering upper respiratory illness and having an adenovirus isolated from their throat washing. Adenovirus types 1 through 10 as antigen were run against each serum pair in a newly developed simplified tissue culture neutralization technic. The test procedure consisted of adding the viral antigen, the serum and a HeLa cell suspension at one time to culture tubes. Neutralization of virus by serum allowed normal HeLa cell growth, while excess virus caused cytopathogenic changes that could be read at 1 or 2 days. Neutralization tests with rabbit type specific antisera failed to show any significant cross-reactions among the 10 types.

Of the 53 persons studied, adenovirus type 3 was isolated from five; type 4 from 25; and type 7 from 23. Four-fold serum neutralizing titer rises were found with the homologous antigen type in all but 2 paired serums. Heterologous rises were found in approximately 20% of the tests with the 10 antigen types. The percentages of heterologous rises with each antigen type ranged from 13 to 44. The most frequent heterologous rises were observed with antigen types 3, 4 and 6.

The presence of neutralizing antibody in the acute serum sample apparently did not predispose to a heterotypic rise. No clear pattern of cross-reactions emerged from the study. It is concluded that there exist immunologic relationships of adenovirus types 1 through 10, as demonstrated by neutralization tests, which are not apparent in rabbit type specific antisera.

Nephrotic Syndrome in Adults

By *Victor E. Pollak, Robert M. Kark, Conrad L. Pirani and Robert C. Muehrcke.* Departments of Medicine, Presbyterian Hospital, Research and Educational Hospital, and Cook County Hospital; Department of Pathology, University of Illinois College of Medicine, Chicago.

In adults the nephrotic syndrome is the clinical expression of many different disease states. It may result from damage primarily to glomeruli, from damage primarily to tubules, or from increased pressure in renal veins, as in renal vein thrombosis and constrictive pericarditis. Clinical differentiation of these causes is difficult. We have studied by percutaneous renal biopsy 67 adult nephrotics, and these studies have reflected a wide range in pathology and etiology.

Subacute or chronic glomerulonephritis was found 26 times. The lesions were membranous in some and proliferative in others, but mixed lesions were frequently found. Diffuse glomerulosclerosis was found in 3 of 16 patients with diabetes mellitus; diffuse and nodular lesions in 7; diffuse, nodular and exudative lesions in 6. Eight patients with SLE had the nephrotic syndrome, and 3 others had the pseudonephrotic syndrome. The lesions of lupus nephritis were more severe in the pseudonephrotics. Primary renal amyloidosis was found once, bilateral renal vein thromboses twice, and once the lesion was undiagnosed. The kidneys were normal in 4 patients studied only after diuresis. With increasing experience it was possible to make exact histologic diagnoses in over 90% of cases, and to forecast, on the degree and type of glomerular damage, the prognosis and the response to treatment with ACTH or corticosteroids.

The histologic appearance of the kidneys was unique in 6 patients. The glomerular capillaries contained an unusually large number of erythrocytes, but the basement membrane and epithelial and endothelial cells of the glomerulus were normal. Severe tubular degeneration and edema of the interstitial tissue were observed. ACTH or corticosteroid administration resulted in an excellent diuresis. During diuresis the tubular epithelium was taller and less degenerated, and the glomerular capillaries contained few erythrocytes. These 6 patients are alive; 3 enjoy excellent health; and none has developed evidence of progressive renal disease.

Nephron Function in Elderly Men Following Bladder Neck Obstruction

By *John B. Wild and Walter M. Kirkendall.* Renal Laboratory, Department of Internal Medicine, and the Department of Urology, State University of Iowa and the Medical Service, V. A. Hospital, Iowa City.

We studied the clearance of sodium para-aminohippurate (C_{PAH}), inulin (C_I), sodium (C_{Na}), potassium (C_K) and chloride (C_{Cl}) in 9 men with prostatism whose ages varied from 65 to 86 (average 75). A total of 19 studies were done. Each patient was tested immediately after decompression. In 3 of them, studies were repeated in the following year to gain information about the functional renal defect and the manner and degree of recovery.

Clearance values are expressed as ml./min./1.73 M^2. C_{PAH} varied from 9 to 307, C_I from 8 to 105 shortly after decompression. Filtration fraction (FF) was high (.23 to .9) in 7 patients, low in 2 (.17, .14), suggesting that proximal tubular function was relatively more impaired than glomerular filtration. This was supported by the fact that renal extraction of PAH in 2 patients was found to be 50% in 1 with C_{PAH} of 44 and 86% in the other with C_{PAH} of 297. C_{Na}, C_K and C_{Cl} were within normal limits in all patients. Three patients were given aminometradine (Mictine), 6 acetazolamide (Diamox) intravenously, and in each electrolyte excretion changed as acutely as in normal persons. This suggests that distal tubular function in the remaining nephrons was adequate to maintain these electrolytes in balance in our patients.

Our results reinforce the knowledge that severe depressions of C_{PAH} and C_I may result from urinary obstruction. They also show that, although these clearances do not improve greatly after relief of obstruction, kidney function was compatible with tolerable health and stationary but slightly elevated blood urea nitrogen levels.

The role of infection, undoubtedly great, could not be separated from those of obstruction and secondary hydronephrosis. Practically all patients on admission had leukocytosis, fever and infected urine and were treated by antibiotics before or after the kidney studies.

Enterodialysis in the Management of Renal Failure: A Metabolic Balance Study with Consideration of the Exchange Rates of Electrolytes and Nonprotein Nitrogen

By *Paul R. Schloerb.* Department of Surgery, University of Kansas School of Medicine, Kansas City; the Research Laboratory and Radioisotope Service, Kansas City V. A. Hospital, Kansas City. (Aided by a grant from the U.S. P. H. S.).

In an attempt to combine the relative merits of intestinal perfusion and extracorporeal hemodialysis for the removal of accumulated toxic metabolites in uremia, a method using perfusion of a closed cellophane tube within the intestinal tract is being developed. An interim evaluation of this method in the normal human, and one terminally uremic patient with correlative findings in nephrectomized dogs, is described in this preliminary report.

A closed cellophane tube is introduced into the intestine. By means of suitable connections, a dialyzing fluid of appropriate electrolyte and osmolar concentration is perfused through the lumen of the cellophane tubing. Exchange rates of radiosodium, radiopotassium and heavy water have been measured between the blood and the lumen of the cellophane tube in both directions in dogs. Metabolic balance studies have included analyses of serum and dialysate fluids for Na, K, Ca, Mg, Cl, P, HCO_3, NPN, BUN, Glucose and pH.

One uremic patient, a 46-year-old man with arteriolonephrosclerosis and malignant hypertension, was terminal despite the usual medical supportive measures and was treated by enterodialysis using 7 feet of $^{27}/_{32}$″ diameter cellophane, introduced into the intestine by jejunostomy under local anesthesia. Intermittent perfusion-aspiration was done, averaging every 150 minutes with a mean volume of perfusate of 400 ml. Six hours after beginning enterodialysis, reorientation and arousal from coma occurred. During the period of 63 hours, when death occurred from myocardial infarction, 50 mEq. of potassium and 14.7 Gm. of nonprotein nitrogen were removed without significant change in other electrolytes. At the shorter perfusion times (60–75 min.), the rates of removal of potassium and nitrogen were double the over-all average, corresponding to daily rates of removal of 36 mEq. of potassium and 12 Gm. of nitrogen, amounts which are in excess of the minimum daily dietary requirement.

KIDNEY

Changes in Body Water Compartments during the Course of Acute Renal Insufficiency

By *Alexander P. Remenchik, James A. Schoenberger and Josephine M. Dyniewicz.* Department of Medicine, University of Illinois College of Medicine, Chicago.

Although changes in body water compartments in acute renal insufficiency have been extensively studied in experimental animals, only a limited number of observations on man has been published. From 1 to 3 simultaneous studies of antipyrine space (AS) and radiosulfate space (RSS) were made in 8 patients with acute renal insufficiency. All patients, after diagnosis, were managed during the oliguric phase by continuous intravenous administration of hypertonic dextrose in water in amounts less than 600 cc./24 hours. In 6 of the patients the initial AS was greater than 60% of the body weight. In 3 patients, who recovered from acute renal insufficiency, there was a marked fall in AS to values within normal limits. In 1 patient studied frequently during the period of oliguria, the initial AS was in the normal range and subsequent changes in AS correlated with changes in body weight. The remaining 4 patients expired before additional studies were done.

The initial RSS was greater than 20% in all of the patients. In the 3 patients who survived, the RSS fell to normal in 2 and to near normal in the other. In contrast to other types of patients, there was no significant loss of radiosulfate from the RSS into sinks other than the urine during the oliguric phase.

The results are in agreement with the conclusions of other investigators that there is an accelerated net catabolic rate in acute renal insufficiency. This increased catabolism of body tissue produces a quantity of water larger than had been previously realized, and the diuretic phase in part represents the excretion of excess quantities of water and electrolytes accumulated during the catabolism of this tissue. If this interpretation is correct, then the therapy of acute renal failure should be modified accordingly.

The Ratio TM/Renal Weight: An Index of Renal Scarring

By *A. P. Crosley, Jr., J. F. Brown, B. Schuster, D. A. Emanuel, H. Tuchman, C. Castillo and G. G. Rowe.* Department of Medicine and Cardiovascular Laboratory, University of Wisconsin Medical School, Madison.

The ratio of the functional tubular mass of the kidney (Tm_{PAH}) to in vivo determined renal weight has been conceived in this laboratory as an index of renal scarring. The following evidence, based on studies of this function in 5 normal subjects and 12 patients with renal disease, is presented in support of this thesis. The normal range for the ratio was 0.16 to 0.26. Patients with chronic glomerulonephritis, where Tm_{PAH} is reduced in proportion to weight as nephrons are destroyed by glomerular damage, had normal values. In contrast, individuals with chronic pyelonephritis, where damage is primarily parenchymal and followed by scar formation, showed a reduction of the ratio in all cases. Diabetics, with nephropathy, where damage may be primarily glomerular and/or parenchymal, had values which were either within normal limits or reduced. Patients with benign nephrosclerosis showed an elevation of this relationship.

These findings, with the exception of benign nephrosclerosis, are therefore in accord with the changes which might be expected from the pathologic findings in these entities. In all types of kidney diseases these observations are in agreement with previous results relating renal oxygen consumption to Tm_{PAH}.

Relation of Renal Vein Pressure to Renal Vascular Resistance and Urine Flow Rate

By *F. J. Haddy, M. Fleishman and J. Scott.* Army Medical Research Laboratory, Ft. Knox, Kentucky.

The role of the absolute level of venous pressure in determining vascular resistance and urine flow rate has been studied in 101 pentobarbitalized laparotomized dogs. In 58 instances, renal blood flow was controlled by interposing a pump in the renal artery. With blood flow rate constant, elevation of renal vein and artery pressures by venous obstruction resulted in an immediate elevation of renal vascular resistance but no change in urine flow rate. Neither value changed in denervated kidneys. During renal artery infusion of acetyl-β-methylcholine and phentolamine, the maneuver resulted in a resistance decrease. The resistance decrease was of borderline significance with phentolamine alone. Progressive elevation of artery pressure and blood flow rate resulted in equal resistance decreases in innervated and denervated kidneys as long as vein pressure was maintained at a low constant value. However, with vein pressure elevated, resistance increased with artery pressure and blood flow rate. In the absence of the pump, urine flow rate immediately decreased upon elevation of vein pressure in both innervated and denervated kidneys. Vein pressure elevation during renal artery infusion of histamine, acetyl-β-methylcholine, phentolamine plus acetyl-β-methylcholine and phentolamine plus physostigmine was associated with urine flow rates

which were well above those with vein pressure normal or elevated in the absence of drugs. The results with phentolamine alone were equivocal.

These findings are interpreted as demonstrating a renal venous-arteriolar reflex having both local and nonlocal components. The urine flow rate decrease induced by vein hypertension may be related to the resistance increase induced by the local component of the reflex. These data also suggest that the apparent "autoregulation" of renal blood flow described by Pappenheimer and others may be more related to activation of the venous-arteriolar reflex than to changes in blood viscosity.

Studies of the Functional Capacity of Autotransplanted Kidneys in Dogs with Comparative Data on the Contralateral (Control) Kidneys

By *Neal S. Bricker, Ralph Straffon, Ed Mahoney and John P. Merrill.* Department of Medicine, Peter Bent Brigham Hospital; the Harvard Medical School, Boston.

Progress in homotransplantation of human kidneys emphasizes the need to thoroughly assess the functional capacity of transplanted kidneys. Moreover, electrolyte excretion data from transplanted (therefore denervated) kidneys may aid in clarifying the disputed role of renal nerves in electrolyte transport. In an experimental approach to this problem, simultaneous functions of the separate kidneys of unanesthetized dogs with divided urinary bladders were measured serially. After control studies, 1 kidney in each animal was autotransplanted (5 dogs) or denervated in situ (2 dogs). Contralateral kidneys served as controls. Follow-up clearances were obtained up to 6 months after surgery.

GFR in all transplanted kidneys decreased markedly initially and thereafter increased, ultimately equalling control (contralateral) values in 2 animals. Regardless of level, GFR increased substantially in transplanted as well as control kidneys following ECF expansion with hypertonic NaCl, hypotonic NaCl or synthetic ECF. ERPF initially decreased in transplanted and increased in control kidneys. Thereafter, values approached pretransplantation levels.

Concentrating ability of transplanted kidneys ($T^{C}_{H_2O}/C_{Cr}$) compared favorably with control kidneys although the response to Pitressin was often delayed.

A relative Na-losing state occurred in most transplanted kidneys. However, 1 animal did not manifest this abnormality, and in 2 others it ultimately disappeared. Similar defects were not apparent in nontransplanted denervated kidneys. Following ECF expansion, Na reabsorption increased with GFR in all kidneys, thereby establishing filtration dependence of Na reabsorption in denervated kidneys. During anesthesia, GFR consistently decreased only in control kidneys, and changes in Na excretion correlated with alterations of filtered loads.

Conclusions: (1) Autotransplantation of dog kidneys is associated with impairment of renal hemodynamics, which is reversible despite normal contralateral kidneys. (2) Permanent denervation is not associated with persisting gross defects in Na transport. (3) Anoxia sustained during transplantation may be causally related to the physiologic abnormalities.

NERVOUS SYSTEM

Frenquel: Studies in Dosage and Duration of Action

By *John T. Ferguson and Thos. G. Allin, Jr.* Department of Medical Research, The Wm. S. Merrell Company, Cincinnati; the Traverse City State Hospital, Traverse City, Michigan.

Patients suitable for treatment with Frenquel are selected on the basis of response to a series of 3 intravenous test doses of 100 mg. given at intervals of 4 hours. In those cases in which behavior is improved, the drug is then administered orally in dosage of 40 mg. 3 times daily with supplemental intravenous Frenquel and other therapeutic measures as indicated.

Therapeutic results with Frenquel have been highly gratifying when patients are selected by this method. On May 1, 1956 a total of 164 patients in the female wards at the Traverse City State Hospital were receiving maintenance doses of 40 mg. 3 times daily by mouth. In all cases a single daily dose of 100 mg. was substituted. In those cases in which behavior was not as well controlled, supplementary doses of 100 mg. were given later in the day as needed. The experiment was terminated after a period of 14 weeks. At that time, of a total of 164 patients, only 76 were maintained adequately on the single daily dose of 100 mg.; 65 had been switched to 100 mg. twice daily, and 23 to 100 mg. 3 times daily.

At the conclusion of the experiment, all the Frenquel patients were put back on 40 mg. 3 times daily, which dosage appears to be as effective as 100 mg. 3 times daily. It is recommended that prior to initiation of Frenquel therapy, all patients

be given a test series of 3 intravenous doses of 100 mg., each at intervals of 4 hours. Those not responding should then be tested with other types of therapy. Those helped by intravenous Frenquel should be put on a maintenance dose of at least 40 mg. 3 times daily by mouth. Other drug psychotherapy and shock may and should be used in combination with Frenquel when indicated.

RESEARCH METHODS

A Graphic Method for the Evaluation of Percentage Differences

By *Henry S. Bloch.* Department of Internal Medicine, State University of Iowa, Iowa City.

Diagnostic and therapeutic considerations in medicine are based to a large extent on collections of cases in which the incidence of certain signs and symptoms in a disease, or the number of patients succumbing to or recovering from an illness, has been counted. The evaluation of such statistics depends on comparison with allied experience. Analytic methods are available for this purpose, but they are rather complex.

This paper describes a simple graph for the evaluation of percentage differences between 2 series of observations.* The abscissa N_A represents the total number of cases in the smaller of the 2 series which are to be compared. The ordinates Δp represent the percentage difference in the incidence of the phenomenon under study between the series. The scales p_0 (or $q_0 = 100 - p_0$) represent the percentage incidence of the phenomenon in both series combined.

The use of the graph may be illustrated by means of a simple example: the mortality in a series of 10,000 cases of *Z* disease was 10%. A physician treats 100 comparable patients with this disease by a new method; the mortality in his series is 4%. Is the success of the new treatment likely to be due to chance alone? To answer this question with the graph one may proceed as follows: (1) Find the value of 100 on the abscissa N_A and draw a vertical line through this point. (2) On the scale of ordinates Δp marked "$1/\infty$" find the value 6 ($=10 - 4$) and draw a horizontal line through this point until it intersects the line through $N_A = 100$. The intercept gives the point Δp, N_A. (3) Find the value of $p_0 = (4 + 1000)/(100 + 10{,}000) \times 100 = 10$ on scales A_1 and A_2 and draw a straight (diagonal) line through these 2 points.

The relation of the point (Δp, N_A) to the diagonal line determines whether or not the difference between the 2 series is "statistically significant." Points on diagonal lines through scales A_1 and A_2 have a level of significance of $p = .01$; points above these lines have a higher level of significance ($p < .01$); those below have a lower level of significance ($p > .01$). Points on diagonal lines through B_1 and B_2 have a level of significance of $p = .05$; points above these lines have a greater, those below have a lesser level of significance.

In the example cited, the difference between the 2 series is not significant at a level of $p = .01$. To test if the difference is significant at the lower level of $p = .05$ draw a line through the points $p_0 = 10$ on scales B_1 and B_2. The point (6100) lies above this line, indicating that the observed difference is statistically significant at a level higher than $p = .05$.

* *Editor's Note:* The graph has been omitted because of physical limitations of the Journal.

Description and Applications of New High-Sensitivity Technics for Study of Serum Lipoproteins by Zone Electrophoresis

By *William Q. Wolfson and Brenton H. Penwarden, Jr.* Department of Medicine and the Laboratory Service, U.S. Army Hospital, Fort Riley; Office of the Regimental Surgeon, Headquarters 18th Infantry Regiment, Fort Riley, Kansas.

Available methods for demonstrating lipoproteins on electropapergrams by staining are insensitive and tend to give diffuse bands and high interfering background, partially corrected at the expense of quantification by washes with mixtures of water and lipid solvents. Such washes are eliminated by a simple modification of postrun Sudan Black B staining, but new technics were required to overcome insensitivity. Prerun staining with Nachtblau reveals serum patterns comparable to postrun Sudan Black B, but without background; both are selective for slow-moving β and zero lipoprotein. Intrarun staining with Nilblau or intrarun staining with Sudan Black B by migration over impregnated paper gives similar patterns which selectively demonstrate the faster lipoalbumin and α lipoproteins. Combined use of prerun Nachtblau and intrarun Nilblau permits demonstration of the full lipoprotein pattern which, under favorable conditions, has included up to 10 discrete bands in normal serum without elevated lipids. Intrarun Nilblau is more reproducible than intrarun Sudan Black B, and the former's background is removable with tap-water; a further major advantage of Nilblau is that, during the run, bands exhibit color

bright gree... through electric blue to brilliant red-yellow.

Such double staining with oxazine dyes long has been known to depend on the fatty acid/neutral fat ratio; colors seen for various serum protein bands are consistent with this view. Since lipoprotein lipase (clearing factor) shifts lipoprotein to faster higher-density components and increases fatty acid/neutral fat ratios, these methods offer a simple method for observing both changes, and their applicability to normolipemic sera increases the possibilities for physiologic studies.

Can Serum Protein-Bound Carbohydrates Accurately Be Detected on Electropapergrams by Stains Employed for Tissue Carbohydrates?

By *William Q. Wolfson and Brenton H. Penwarden, Jr.* Laboratory Service and the Department of Medicine, U.S. Army Hospital, Fort Riley; Office of the Regimental Surgeon, Headquarters 18th Infantry Regiment, Fort Riley, Kansas.

Serum electropapergrams of normals and patients were stained for protein with bromphenol blue or amidoschwarz, and compared with duplicates stained with Schiff reagent, toluidine blue, acid crystal violet and/or mucicarmine following no oxidation, periodic acid, bromine water or periodic and bromine. Isolation data show about half of serum protein-bound carbohydrate in α globulins with virtually none in albumin. Reproduction of this pattern was the standard for evaluating staining technics, and none were successful. Schiff is capricious and highly subject to artifacts. Untreated strips with presumably masked aldehydes stain strongly. Oxidation increases are chiefly in low-carbohydrate regions. Peak Schiff/protein ratios usually are not in α globulin. Toluidine blue stains untreated strips poorly, acid crystal violet well. Both increase intensity and toluidine blue, also metachromasia with periodate, but more strikingly with bromination. However, bromination or periodate virtually abolishes mucicarmine staining. Except for one myeloma component, all fractions showed toluidine/protein ratios between 0.85 and 1.35, indicating nonselectivity.

There exists a demonstrable and real discrepancy, as yet unexplained, between Rice's results with acid crystal violet and ours. Such results suggest that each of these special stains colors a chemically different component. However, if each stain is used under conditions of maximal staining, the pattern always resembles the general protein pattern in locus and relative intensity of bands. These res... do not support the view that any of the in... procedures are reliable indices of serum pr... bound carbohydrate. The simplest, and pe... strongest objection, however, is that cur... ethods make the implicit assumption that 3 μg. of carbohydrate (about 0.2 μg. of heteropolysaccharide reactive aldehyde) distributed over 1 cm.^{2} of paper will give brilliant staining by methods which are not particularly notable for high sensitivity or freedom from background.

A Rapid Colorimetric Method for the Determination of Oxygen Saturation of Whole Blood by Reflectance

By *William Meltzer and George A. Saxton, Jr.* Respiratory Center, Department of Preventive Medicine, University of Illinois College of Medicine.

A simple, inexpensive method for determining oxygen saturation of human blood, unaffected by hematocrit changes, seemed desirable. Therefore, an abridged reflectance spectrophotometer (Color Eye), employing a broad-band red (X) filter (520–700 mμ), was used to compare blood samples of known saturation with a series of color comparators, to establish the latter as standards for future comparisons with bloods of unknown saturation. It was then possible to make visual comparisons in a simple black box using light passed through an X filter, to determine oxygen saturation quickly and inexpensively.

The following facts were established: (1) Fully saturated blood from different normal individuals is the same color as indicated by the Color Eye and visual color comparators. Twenty of 24 bloods from different individuals, when equilibrated with room air, gave identical Color Eye readings. The other 4 (all indicated lower saturations) were found to have elevated hematocrits (52–68). (2) Hematocrit is not a factor affecting reflectance within certain limits. Blood from individuals with normal hematocrits did not show different reflectance when the RBC's were concentrated by centrifugation or diluted by addition of plasma, between 15 to 75 hematocrit. (3) The color of whole blood at a given saturation does not vary as a function of oxygen tension. Equilibrating blood with room air and with 30, 40, 60, 80 and 100% oxygen gave identical Color Eye readings.

On the basis of these studies a simple reflectance method for the determination of oxygen saturation of freshly-drawn arterial blood in a syringe has been established. A blood sample is compared visually with commercially available calibrated color standards to a degree of accuracy of plus or minus 1–2% in the range of 70–100% saturation. The clinical practicability of the method is much broader than those which require a technician and a laboratory instrument, since the cost of a light source, X filter, black box and color comparators does not exceed $100, and the method can be used without special training.

RESPIRATORY SYSTEM

A Simple Inexpensive Method for Determination of Vital Capacity and Maximal Breathing Capacity in Normal Subjects and in Patients with Lung Disease

By *Eugene W. Worton.* Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City.

The purpose of this paper is to present a method for measuring vital capacity and maximal breathing capacity (MBC) in normal people and patients with lung disease utilizing simple and inexpensive equipment, and to compare the accuracy of values obtained with this equipment with those obtained using standard equipment. The apparatus consists of a mouthpiece, an Edison unidirectional valve, a 30-inch piece of corrugated rubber tubing and an American Meter Company gas meter. Values are read from a dial on the face of this meter. No recording apparatus is necessary. Cost of the equipment is less than $100. To perform the tests the subject inspires room air and expires through the meter.

Vital capacity measurements were made using this equipment and a Benedict Roth spirometer (standard method). Twenty normal subjects and 10 patients with lung disease were studied. Mean values in normal subjects were 4.54 L. by the gas meter method and 4.55 L. by the Benedict Roth method; in patients with lung disease, 3.23 L. by the gas meter and 3.04 L. by the Benedict Roth method.

MBC was measured with the apparatus described and also by the standard method in our laboratory, utilizing a low resistance valve and a Tissot spirometer. The same 30 subjects were studied. Mean values in normal subjects were 121.7 L. by the gas meter method and 141.4 L. by the Tissot method; in patients with lung disease, 41.3 L. by the gas meter method and 49.7 L. by the Tissot method.

Measurement of vital capacity is extremely accurate by this method. MBC values were lower by this method than by the standard method, which is compatible with the known higher resistance in the gas meter system. The method is sufficiently accurate to be used by practicing physicians, and combines economy and simplicity.

The Inaccuracy of the Water-Filled Spirometer in the Measurement of the Maximum Beathing Capacity

By *William W. Stead, Herbert S. Wells, N. L. Gault and John Ognanovich.* V. A. Hospital, Minneapolis.

Bernstein has shown that the water-filled spirometer may record anywhere from 50–140% of the volume of air that is pumped into it during determination of maximal breathing capacity, depending upon the characteristics of the spirometer and the rate and depth of the respiration. We have studied both the 9 and the 13.5 L. respirometers made by Collins, and have obtained curves of frequency response that are similar in form to that given by Bernstein for the Knipping spirometer. The purpose of this paper is to illustrate some of the causes for the recording errors observed.

At rates of breathing and air flow that are involved in basal breathing the spirometer records accurately (response of 100%). At frequencies of 80–90/min. the spirometer records less than the actual volume (response of 90%). At greater frequencies the spirometer overshoots (response progressively greater than 100%). The inaccuracy of the 9 L. spirometer is greater than that of the 13.5 L. spirometer.

Analysis of the physical reasons for the discrepancies has revealed the following: (1) The early under-response is due to the motion of the water as it is forced up and down by the rapidly changing pressures. This effect can be lessened considerably by the use of a light plastic bell which fits close to the inner drum of the spirometer. (2) The later and progressively greater overshoot of the recording pen is due to a combination of factors, the greatest of which are the inertia associated with the weight of the metal bell and its counterweight, and the snapping and slacking of the chain which connects the moving bell with the recording pen. These defects were mitigated by mounting the writing pen directly on the light plastic bell. We are presently working on other causes of inaccuracy in spirometer recording, and on a design for a better instrument.

Nitrogen Clearance Rates of Right and Left Lungs in Different Postures

By *Glen A. Lillington, Ward S. Fowler, R. Drew Miller and H. Frederic Helmholz, Jr.* Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Study of total expired gases has shown that nonuniformity of alveolar ventilation occurs normally and is increased in many pulmonary diseases, but the responsible mechanisms and spatial locations are uncertain.

Alveolar ventilation of individual lungs was studied by use of bronchospirometric catheter and continuous measurement of concentration of nitrogen and flow rate of gases expired during inhalation of oxygen. Right and left lungs of 5 normal men in the supine and both lateral decubitus positions were studied. Nitrogen clearance curves of multiple exponential form were found, and their analysis showed that uneven alveolar ventilation of similar magnitude was present in all lungs in all postures. In the supine position, tidal volume, dead space and functional residual capacity were greater for the right lung than the left, but turnover rates of al-

veolar gases and the relative magnitude of slowly and rapidly ventilated regions of both lungs were similar. In lateral decubitus positions, the ventilatory pattern of the dependent lung was similar to that of the supine position. The superior lung had an increased functional residual capacity and decreased tidal volume. Alveolar turnover rates of both rapidly and slowly ventilated components of a lung were only about half as great when it was in the superior position as they were in the same lung when the patient assumed the supine position.

Relative alveolar turnover rates of right and left lungs varied with certain postures, an indication of the existence of regionally uneven ventilation of an anatomically variable type. Uneven alveolar ventilation of undetermined nature also occurred within normal single lungs.

The Influence of Plasmin upon Experimental Pulmonary Emboli and Pulmonary Infarcts

By *Paul Ruegsegger, Irwin Nydick, John S. LaDue and Eugene E. Cliffton.* Memorial Center for Cancer and Allied Diseases, New York City.

The effects of plasmin, a fibrinolytic enzyme, were studied during different phases of the evolution of pulmonary infarcts in rabbits. A method was devised whereby pulmonary infarction could be produced consistently following embolization. Plasmin was administered intravenously in varying dosages at different times following embolization, and its influence upon the animals' course was studied.

Polyethylene catheters were threaded into the right heart of rabbits via the jugular vein. Preformed clots of different sizes were injected into these catheters in a group of 17 control rabbits and a group of 15 "treated" rabbits.

Sixteen of the 17 control rabbits revealed pulmonary infarction at autopsy, usually extensive in degree. Four of these rabbits had died within 24 hours after embolization from shock or acute right heart failure.

Plasmin was administered to the treated group in doses of 6 to 9 mg./Kg. body weight. All of the plasmin-treated rabbits survived. None of the 5 rabbits treated within 40 minutes after embolization showed infarction at autopsy. Of 6 rabbits treated within 4 to 24 hours, the lungs were normal in 3, and single small residual infarcts were seen in the other 3. Two animals treated after 40 hours showed somewhat larger areas of infarction. Two animals were treated promptly after 2 separate series of embolization. Both survived, 1 with clear lungs and the other with 2 small infarcts, whereas the control rabbit died promptly after the first embolization.

Endobronchial Involvement in Systemic Sarcoidosis

By *Gordon L. Snider and S. Allen Mackler.* Michael Reese Hospital, Chicago.

Sarcoidosis has been reported to involve virtually every organ and tissue of the body. The lung parenchyma is commonly involved in systemic sarcoidosis, but bronchial involvement has been reported infrequently. In 1952 a patient presenting with the clinical picture of bronchial asthma was found to have systemic sarcoidosis with endobronchial infiltration by the disease process.

Since that time, approximately 40 cases of sarcoidosis have been thoroughly studied, including ventilatory function studies and biopsy of enlarged superficial nodes or scalene lymph nodes. Bronchoscopy and routine bronchoscopic biopsy were performed on these patients. Although the mucosa appeared grossly normal in most instances, over half of the biopsy specimens showed granulomatous endobronchitis consistent with sarcoidosis. Ventilatory function studies frequently showed mild degrees of reversible airway obstruction.

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